“Improving poisoning management through

(A) A pilot study to evaluate the use of the triple cholinesterase test in Organophosphorus poisoning and

(B) A study on the changing profile of poisoning presenting to a tertiary care centre in South India”

A dissertation submitted in partial fulfilment of the rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai, to be held in April 2016

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DECLARATION

This is to declare that this dissertation titled “Improving poisoning management through (A) A pilot study to evaluate the use of the triple cholinesterase test in Organophosphorus poisoning and (B) A study on the changing profile of poisoning presenting to a tertiary care centre in South India” is my original work done in partial fulfilment of rules and regulations for MD General Medicine examination of the Tamil Nadu Dr.M.G.R Medical University, Chennai to be held in April 2016.

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This is to certify that the dissertation entitled, "Improving poisoning management through (A) A pilot study to evaluate the use of the triple cholinesterase test in Organophosphorus poisoning and (B) A study on the changing profile of poisoning presenting to a tertiary care centre in South India" is a bonafide work done by

Dr. Sandhya Suresh

towards the partial fulfilment of rules and regulations for MD General Medicine degree examination of the Tamil Nadu Dr.M.G.R Medical University, to be conducted in April 2016.

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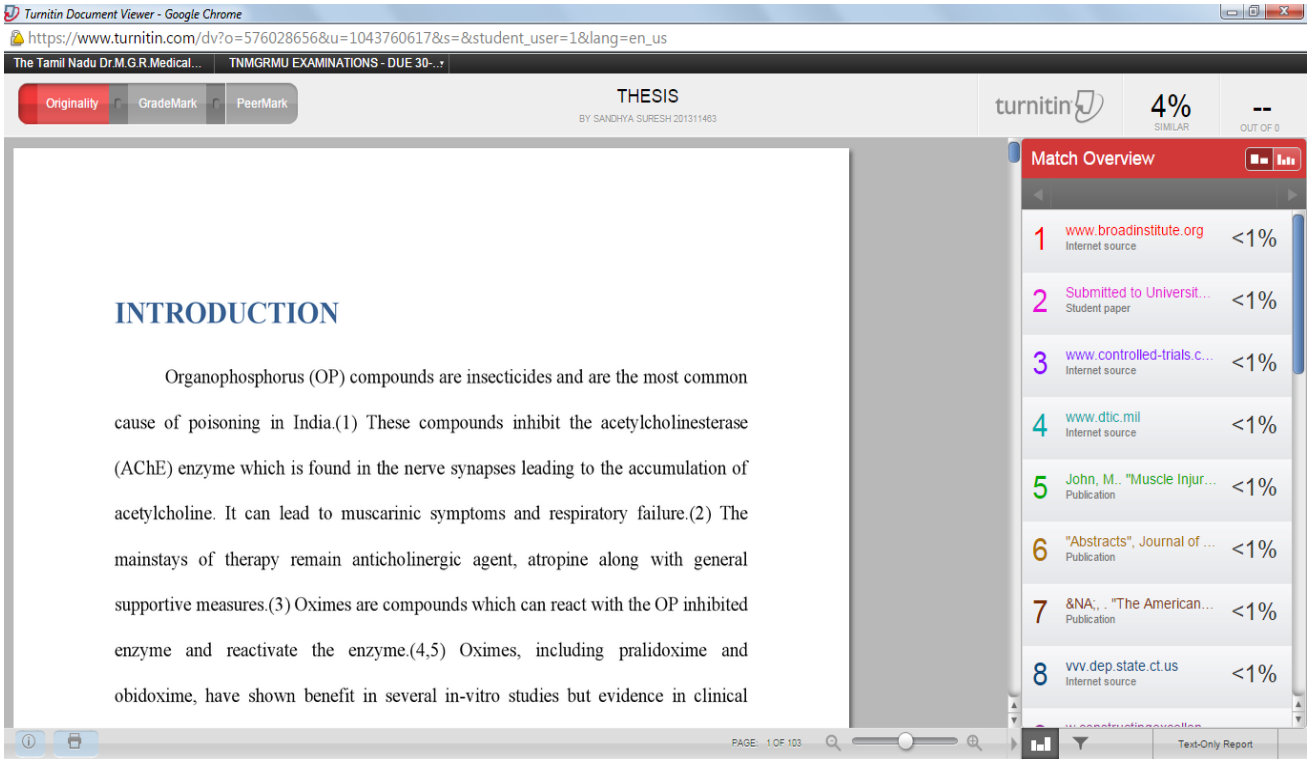

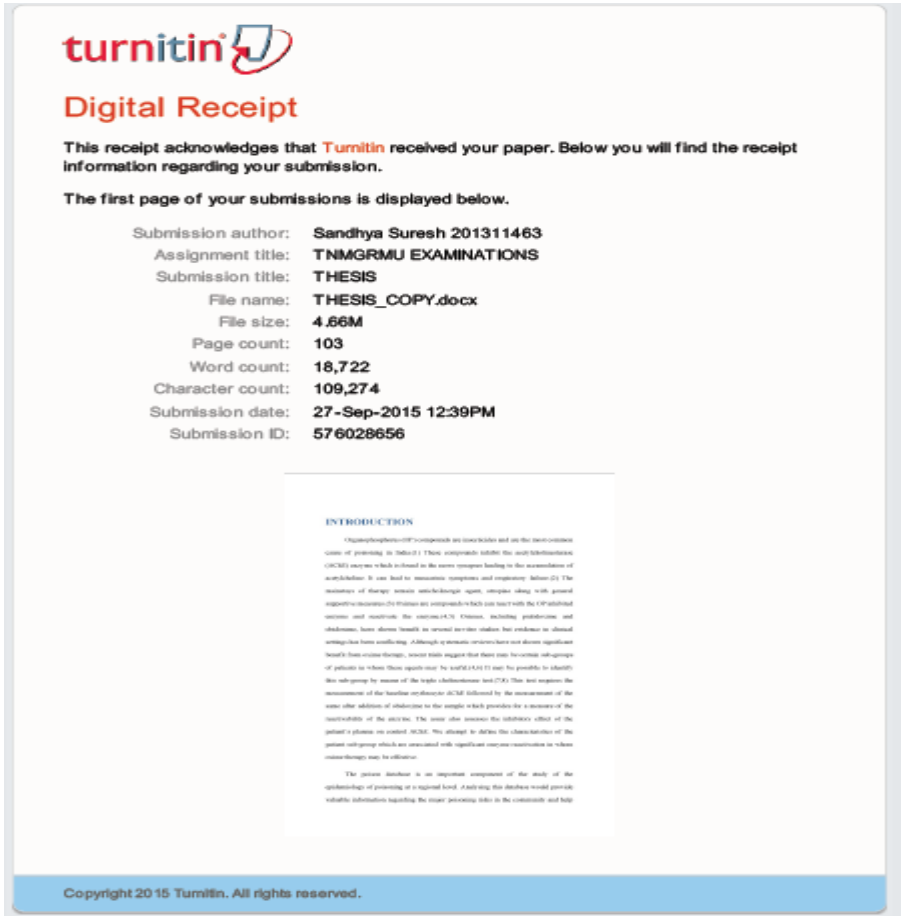

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INTRODUCTION

Organophosphorus (OP) compounds are insecticides and are the most common cause of poisoning in India.(1) These compounds inhibit the acetylcholinesterase (AChE) enzyme which is found in the nerve synapses leading to the accumulation of acetylcholine. It can lead to muscarinic symptoms and respiratory failure.(2) The mainstays of therapy remain anticholinergic agent, atropine along with general supportive measures.(3) Oximes are compounds which can react with the OP inhibited enzyme and reactivate the enzyme.(4,5) Oximes, including pralidoxime and obidoxime, have shown benefit in several in-vitro studies but evidence in clinical settings has been conflicting. Although systematic reviews have not shown significant benefit from oxime therapy, recent trials suggest that there may be certain sub-groups of patients in whom these agents may be useful.(4,6) It may be possible to identify this sub-group by means of the triple cholinesterase test.(7,8) This test requires the measurement of the baseline erythrocyte AChE followed by the measurement of the same after addition of obidoxime to the blood sample which provides for a measure of the reactivability of the enzyme. The assay also assesses the inhibitory effect of the patient's plasma on control AChE. We attempt to define the characteristics of the patient sub-group which are associated with significant enzyme reactivation in whom oxime therapy may be effective.

The poison database is an important component of the study of the epidemiology of poisoning at a regional level. Analysing this database would provide valuable information regarding the major poisoning risks in the community and help

in improving management of common poisonings.(9,10) This study was designed to describe the changing profile of adult poisonings presenting to Christian Medical College (CMC), Vellore. We attempted to describe the relative proportions of different groups of poisons including pesticides, plants and drugs along with their outcomes and their temporal profile.

AIM

To study the role of triple cholinesterase test in the management of Organophosphorus (OP) poisoning and the changing trends in the profile of poisoning presenting to a tertiary care centre in South India.

OBJECTIVES

(A) TO EVALUATE TRIPLE CHOLINESTERASE TEST IN ORGANOPHOSPHORUS POISONING

1. To validate a battery of the following tests for use in patients with organophosphorus poisoning to determine applicability of oxime therapy – RBC acetylcholinesterase(AChE), Plasma butyrylcholinesterase(BChE), enzyme reactivating potential of obidoxime ex-vivo and Inhibitory activity of patient plasma – and to determine their temporal profile
2. To correlate these test results to the clinical characteristics of acute organophosphate poisoning including type of organophosphate, time to presentation, severity of poisoning and development of complications
3. To determine the clinical characteristics of patients with organophosphorus poisoning in whom oxime therapy would be useful.

(B) TO STUDY THE CHANGING PROFILE OF POISONINGS IN CMC, VELLORE BETWEEN 2009-2014 USING THE POISON DATABASE

1. To study the changing profile of pesticide, plant poisoning and drug overdose during the period 2009-2014.
2. To study the changing demographics of patients who present with poisoning and comparison of accidental poisoning and deliberate self-harm due to pesticide, plant poisoning and drug over dose.
3. To study the overall mortality and poison specific mortality in relation to pesticide, plant poisoning and drug overdose.

A REVIEW OF LITERATURE

EPIDEMIOLOGY OF POISONING

The WHO estimates 800,000 deaths due to suicides each year with many more attempted suicides. Suicide accounted for 1.4% of deaths in 2012 with over three-fourths of the cases occurring in the low and middle-income countries.(11) Globally, it was also the second most common cause of death in women between 15 to 29 years of age. India's suicide rate falls in the bracket of >15 per 100,000 population and falls in the category of countries with high suicide mortality as shown in the representative map below.(11) In 2012, a WHO appraisal estimated that 2.6% of deaths in India could be attributed to deliberate self-harm and this rate was on a rising trend.(12)

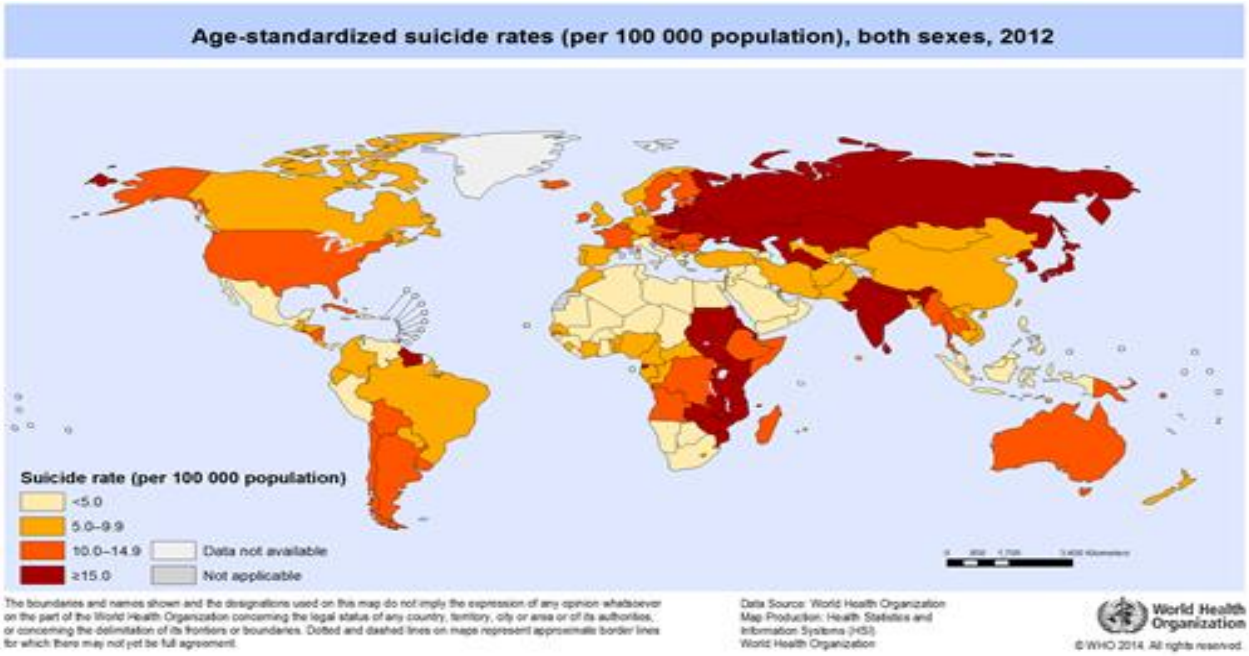


Figure 1: Age standardized suicide rate (per 100,000 population), 2012 (11)

As per the 2011 census in India, *"Injury, Poisoning & Certain Other Consequences of External Causes"* accounted for 7.8% of all medically certified deaths and were the leading cause of mortality in the age group of 15-24 and 25-34

years accounting for 27.5% and 23.5% respectively in these groups.(13) This signifies the vulnerability of the adolescent age-group and youth to deaths due to deliberate self-harm. Poisoning due to drugs, biological substances and non-medicinal substances caused 1.5% of all deaths in India in 2010. 71.9% of these deaths occurred in the working age-group of 15 to 54 years of age. Data on poisoning has been widely fluctuating over the years and between different regions depending on the availability of different poisons and the efficacy of reporting systems. The above numbers may be an under-estimation as mortality statistics in India are limited by poor reporting systems and an evaluation in the census showed that of the total registered deaths in the country, only 20% were medically certified.(13) The Million death Study found that a 15 year old in India had a cumulative risk of 1.2% of dying by suicide before 80 years of age with Tamil Nadu being in the highest risk bracket of >2% risk (2.2%).(1,14)

EPIDEMIOLOGY OF PESTICIDE POISONING IN THE WORLD

Pesticides, although an important component of agricultural practice as a defence against pests, are highly toxic and poisoning with these agents is a major problem in the developing world, especially in the rural regions. There are an estimated 3 million cases of pesticide poisoning worldwide every year resulting in 250,000 deaths and leaving thousands more in morbidity and disability.(15) Of these cases, one-third are accidental and two-thirds are due to deliberate self-harm.(16) Most of these cases occurred in India, China, Vietnam and Sri Lanka.(15) 20.7% of suicides occurring in south-east Asia are due to pesticides compared to 3.9% and 4.7% respectively in Americas and Europe.(17)

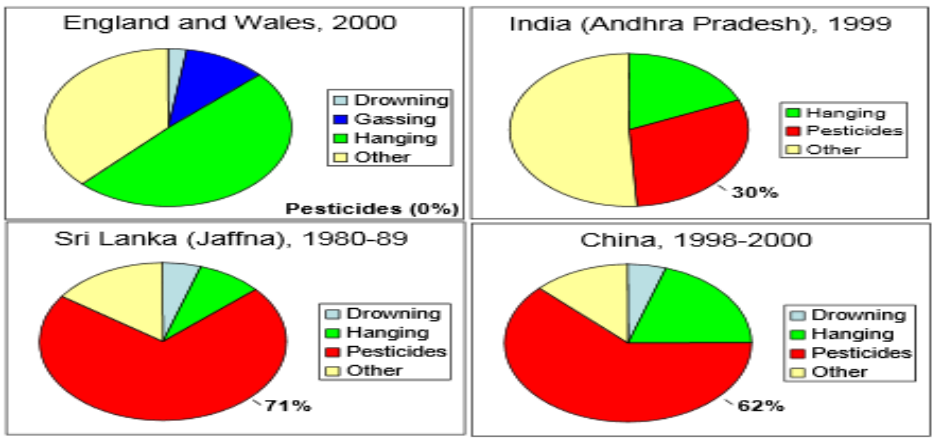


Figure 2: Pesticides as a cause of fatal self-harm in Asia(15)

PESTICIDE POISONING – INDIAN SCENARIO

Poisoning, mainly with OP compounds, account for about half the suicides in the country which was estimated as 187,000 deaths every year in the million death study.(1) A study done in the state of Maharashtra over a period of five years quantitated organophosphorus as the causative agent in 43% of fatalities due to poisoning.(18) What makes pesticide poisoning a popular method for suicide is the easy availability of these compounds especially in countries with an agrarian economy where these toxic compounds can be bought "over the counter" and lack of knowledge about their adverse effects.(19,20) Some studies have also shown that chronic as well as acute exposure to organophosphorus can induce affective disorders which may in turn increase the suicidal intentions.(21) This led to the recommendation from several authors to increase regulations and restrict access to pesticides especially organophosphates as part of suicide prevention strategies.(1,16,17,22)

EFFECT OF RESTRICTIONS ON TOXIC PESTICIDES

One major contributor to the changes in suicide mortality in high-income countries is the change in the lethality and in the availability of the suicide methods

commonly used.(23) A study in Sri Lanka showed that after the restriction of toxic pesticides, there was a fall in the mortality rates due to poisoning without impacting the rate of deliberate self-harm.(24) In Sri Lanka, the most toxic organophosphates, namely parathion, methylparathion, monocrotophos and methamidophos were banned by 1984 and all WHO class I toxic compounds were banned by 1995. There was a transient rise in suicide mortality in the transitional period up to 1995 as endosulfan use increased after which endosulfan was also banned in 1998. There was a progressive drop in suicide rates thereafter with rates becoming almost halved in 2005 compared to 1995 indicating that the restriction of toxic pesticides may have limited the mortality.

It is known that having access to easily available and highly toxic pesticides increases the rate of deaths due to intentional poisoning. This was proved when the change to benzodiazepines from barbiturates led to a fall in mortality due to pharmaceutical poisonings.(25) The WHO and FAO recommended progressive ban on the class Ia and Ib (extremely and highly hazardous) pesticides and to use alternatives for pest management.(26,27) As per these recommendations, several pesticides have been restricted or banned from use in India. For example, Monocrotophos is banned from use in vegetable crops. Some of the changes suggested to reduce access to toxic pesticides include restricting pesticide use to only less toxic compounds and subsidizing the costs of these alternative pesticides. Education of farming communities and better poisoning management practices have also shown to be effective in lowering the poisoning mortality.(28) *Eddleston et al* propounded the

development of a “minimum pesticides list” including permissible pesticides which are less dangerous.(25)

However, there is no system in place in India to implement the regulations on pesticide use.(29) Newer and less toxic pesticides are being progressively introduced into the market in India. We know that pesticide restriction has not been effective in India. These changes of introduction of newer pesticides and lack of restriction may ultimately impact both the profile of poisonings and deaths due to them in the country.

POISONING IN CMC, VELLORE – A SHIFT IN PROFILE?

A surveillance study was done between July 2009 to June 2011 in CMC using the poison database.(30) It included 1164 patients with poisoning of whom 52.7% were men with 29.4 years being the mean age. 97% of them were due to deliberate self-harm with pesticides accounting for 53.4% of which organophosphates were implicated in 54% of the cases. Drugs accounted for 28.3% and plant poisons for 9.5% of all the poisoning cases. During this period, 70% of all the organophosphates belonged to the highly toxic class I category with a case fatality rate of 8.4%. Organophosphates were also the most common cause of death due to poisoning causing 29.1% of all the poisoning deaths. Considering the high mortality rates and the high prevalence of the highly toxic group of pesticides, it called for the regulation of these pesticides as has been done in several other countries. It also established the use of poison data base from the CMC Poison centre in monitoring trends in poisons.

A study was initiated in CMC, Vellore to examine the pathophysiology of shock in the Oduvanthalai poisoning. *(Pathophysiological mechanism of*

cardiovascular toxicity in Oduvanthalai or Cleistanthus collinus poisoning. IRB Min. No 8944 dated 7.07.2014) Oduvanthalai poisoning is the most common plant poison in Vellore district with a mortality of about 30%.(31) This study was started in view of the lethality and lack of knowledge regarding the pathophysiology and the absence of an effective treatment. There was found to be a sharp decline of Oduvanthalai poisoning which may reflect a changing epidemiological profile. Oduvanthalai poisoning is a traditional method of deliberate self-harm, almost exclusively among rural community, more common among women and older people. Although the planned study was not possible, the decline in cases of Oduvan poisoning may reflect preference of other poisons such as pesticides and drugs among younger people or a change in referral pattern. There has also been a perceptible shift in the type of pesticides consumed with greater prevalence of less toxic pesticides and mixed pesticides. Whether this has impacted the mortality due to poisonings has to be evaluated through this study of the poison database.

POISON INFORMATION CENTRES & TOXICOVIGILANCE

Many studies have also noted the gross under-reporting of pesticide poisoning in India with one study estimating seven times higher mortality than the reported rates.(17) This indicates the utility of maintaining a poison database in every hospital to determine the exact magnitude of the problem.(29) This led to the WHO advocating the establishment of poison centres that specialize in prevention, diagnosis and management of poisoning. (32) They also maintain databases that provide data about the type of agents used for poisoning, the means by which they were obtained and the effects of the poison which could direct further preventive measures. They help in

identifying and evaluating existing and emerging toxic hazards in the community which is now known as toxicovigilance.(10,32) Toxicovigilance entails detailing the toxic risks in the community along with reporting to the appropriate health authorities and developing measures to reduce the emerging exposure risks.(9,33) The first such poison information centre in the country was established in the department of Pharmacology in All India Institute of Medical Sciences, New Delhi in 1995 and its functions include advice on management of poisoning, toxicovigilance, training measures for medical personnel and prevention of poisoning.(34) This could guide policy-makers in public health settings especially in decisions regarding regulation of pesticide use and also in the development of a comprehensive poisoning prevention and management programme.(35) In our local region, Poison control centres have been established in Madras medical College and Amrita Academy of Medical Sciences.

Medicine Unit I has adopted the function of a poison control centre with functions including clinical services, training, documentation, research, community outreach and advocacy. It has been prospectively collecting a poisoning data base from 2009. We will use this data base to examine the changing hospital based epidemiology of poisonings at Christian Medical College. The goals of this data base, conforming to the WHO recommendations, are as follows:

1. To monitor the profile of poisonings and mortality
2. To audit the care of poisoning patients and provide feedback to clinicians
3. Provide the basis for guidelines and improvement in clinical care
4. For public health policy advocacy in preventing poisons.

The main role of our CMC, Vellore Poison Control Centre is to collect, collate and analyse information about poisoning in the community on a regional basis. It is important to determine the magnitude, pattern and outcomes of common poisonings in the region. Analysis of this database may reflect the changing profile of poisoning in the community and guide preventive measures and also in the early diagnosis and management of these common poisonings.

ORGANOPHOSPHORUS COMPOUNDS

Organophosphorus compounds are popular options for suicide attempts due to their easy availability in many Indian households in view of their utility as pesticides. Several studies in India have implicated these compounds as the most common cause of deliberate self-harm in the country especially among the farming community.(36)

STRUCTURE OF ORGANOPHOSPHORUS COMPOUNDS

The pathogenesis of the toxicity of organophosphorus compounds in humans was first recognised in the 1930s in Germany. Its structure consists of a tetra-substituted phosphorous centre with a double bonded oxygen or sulphur atom and a leaving group with two other substituents depending on the compound subclass.(37)

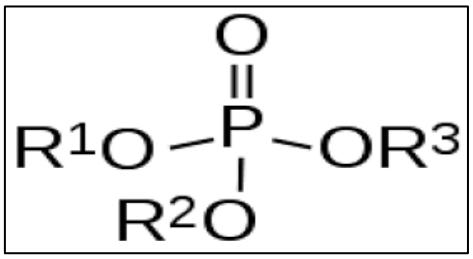


Figure 3: Chemical structure of organophosphorus molecule (38)

CLASSIFICATION OF ORGANOPHOSPHORUS COMPOUNDS

The organophosphorus compounds can be classified in various ways as follows(2):

1. **Depending on whether they are pro-poison or active poison**
   a) Thion (pro-poison) – Parathion, Chlorpyrifos, Dimethoate – with sulphur atom attached to phosphate atom (P=S) and need conversion by CYP450 in gut wall or liver to active oxon (P=O) for clinical effects
   b) Active oxon form – Profenofos, Dichlorvos – faster acting as they do not require activation
2. **WHO classification based on rat LD50**(26)
   - Class Ia (extremely hazardous) – Parathion, Methyl-parathion
   - Class Ib (highly hazardous) – Dichlorvos, Dicrotofos, Monocrotofos, Triazophos
   - Class II (moderately hazardous) – Chlorpyrifos, Diazinon, Fenthion, Quinalphos
   - Class III (slightly hazardous) – Methylchlorpyrifos, Malathion

- This classification is based on rat toxicity and was applied for occupational use and not for deliberate self-harm. Therefore, although class II compounds may be less toxic than their class I counterparts, in the setting of self-poisoning, these are highly lethal compounds.

3. **Chemical structure** (depending on the structure of R1 and R2 – as in chemical structure of organophosphorus molecule – *Figure 1*(38))
   a. Diethyl – Chlorpyrifos, Quinalphos, Parathion
   b. Dimethyl – Methyl-parathion, Monocrotophos, Fenthion, Dimethoate
   c. S-Alkyl – Profenofos, Methamidophos
4. **Lipid solubility** – Higher the lipid solubility

- Delayed onset, longer acting
- Sudden release of compound resulting in sudden severe cholinergic crises and respiratory arrest
- Fention, Dichlorfenthion, Parathion, Chlorpyrifos – more lipid soluble among the organophosphorus compounds

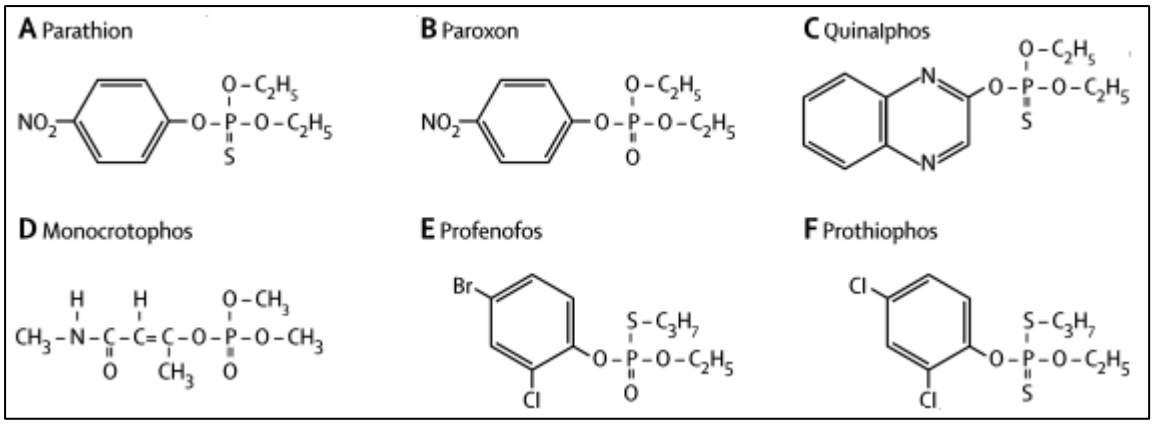


Figure 4: Chemical structure of organophosphorus pesticides – A,B,C are diethyl, D is dimethyl and E,F are S-alkyl compounds; A,C,F are thions and D,E are oxons (3)

TOXICITY AND CLINICAL MANIFESTATIONS

These compounds inhibit the synaptic enzyme acetylcholinesterase (AChE) which breaks down acetylcholine leading to accumulation of acetylcholine at the synapses and overstimulation of muscarinic and nicotinic receptors of the central and autonomic nervous system and neuromuscular junction. AChE is an esterase enzyme that has two binding sites, namely the anionic and esteratic sites. OP compounds covalently react with the serine moiety in the estaratic site producing a serine-phosphoester bond due to this nucleophilic attack. This inactivates the enzyme preventing esteratic cleavage of AChE and causes accumulation of acetylcholine at the nerve endings. Acetylcholine a neurotransmitter found in the neuromuscular

junction, pre-ganglionic autonomic nerves as well as the post-ganglionic parasympathetic nerves. This is similar to butyrylcholinesterase which is present in plasma. The acetylcholine accumulation leads to generalised, prolonged central and peripheral post-synaptic stimulation.(39) Nicotinic cholinergic receptor activation results in involuntary contraction of skeletal muscles manifested as fasciculations and complete depolarisation block which in turn causes flaccid paralysis. Muscarinic receptor activation activates the secretory glands resulting in the commonly described SLUDGE symptoms of organophosphorus poisoning which is composed of 'Salivation, Lacrimation, Urination, Diaphoresis, Gastrointestinal upset and Emesis'. Other muscarinic effects include bronchorrhoea, bronchoconstriction and miosis along with bradycardia. The central nervous system effects of the accumulated acetylcholine include disorientation, anxiety and seizures succeeded by loss of consciousness with respiratory arrest.(40) Death may result due to cardiorespiratory arrest or due to prolonged seizures, especially in the nerve agents such as sabin and soman.(39,41) The severity of these clinical manifestations depend on several factors including dose ingested, onset of action of the compound, rate of absorption and stability of the formulation. The oxons which do not require conversion to active form will have faster action but for the thions, onset of toxicity will vary depending on the rate of their activation. For example, parathion which gets activated rapidly will act faster than dimethoate which is a slow inhibitor of AChE as its conversion to omethoate, the oxon form, is slower.(42) The clinical profile of organophosphorus poisoning has been graded based on severity by Namba in his 1971 study on parathion and its manifestations as described below.(43)

ENZYME REACTIVATION AND OXIMES

AChE inhibition caused by OPs last longer than that caused by Carbamates. Organophosphorus compounds react with the enzyme at the ester site and the bond stability is dependent on the molecular structure of the OP compound. Hydrolysis may occur spontaneously with methyl and ethyl compounds but may be hindered in compounds with larger alkyl groups which results in a phosphorylated enzyme which is inactive. Spontaneous reactivation of the enzyme occurs slowly with half-life of 0.7 hours for dimethyl compounds and 31 hours for diethyl compounds. A process of 'ageing' is said to occur when one of the alkyl groups is removed from the phosphate group of the inactive enzyme, leaving a hydroxyl anion group attach to the phosphorous at the ester site. Once aged, It is left with a phosphate that is negatively charged and it cannot be further regenerated by a nucleophile with a negative charge such as oximes (as described in *figure 2*).(4,5) When there is a greater quantity of inactivated compound, such as in compounds with sow spontaneous regeneration, there is a higher rate of ageing. The rate of ageing is faster for dimethyl compared to diethyl compounds with half-life of 3.7 hours and 31 hours respectively resulting in therapeutic windows of 13 and 132 hours respectively. After 7 hours of poisoning, 75% of enzyme is not reactivable in dimethyl OP poisoning. In case of S-alkyl compounds, There is almost immediate 'ageing'.(2)

Oximes are nucleophilic compounds that react with and transfer the phosphoryl group from the enzyme to itself. (4) The basic oxime group catalyses the hydrolysis of the phosphodiester bond between the cholinesterase enzyme and the organophosphorus compounds and thereby frees the active site of the enzyme.(44)

They enhance the rate of reaction 2 described in the above figure 4 and reduce the amount of inactivated enzyme which is available for the ageing process.

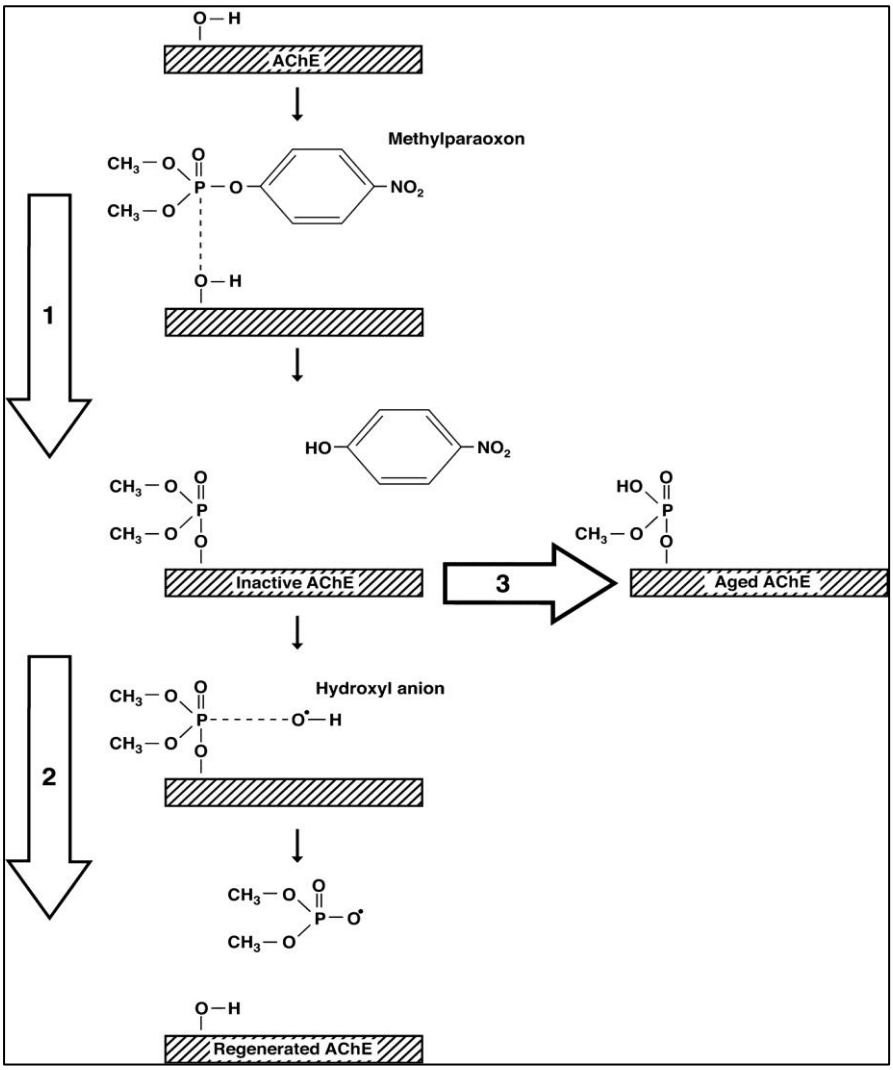


Figure 5: Reaction of OP with AChE (4)

There are four main oximes which have been used in clinical trials and clinical settings – pralidoxime, obidoxime, Hl6, and Hlo7. Obidoxime is forty, nine, and three times more potent than Hl6, Hlo7, and pralidoxime, respectively.(45) More oxime is required to reactivate a dimethyl compound than a diethyl compound. Dimethyl compounds need 20 times more pralidoxime than obidoxime and diethyl compounds need 7 times more pralidoxime than obidoxime.(46)

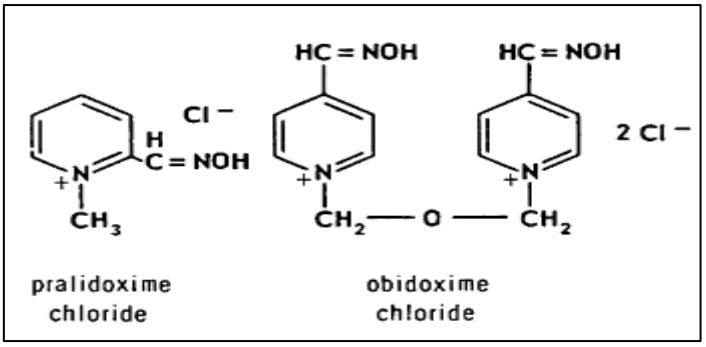


Figure 6: Structural formulae of pralidoxime chloride and obidoxime chloride (47)

SYNDROMES OF ORGANOPHOSPHORUS POISONING

1. **Acute cholinergic crises:** Predominantly muscarinic symptoms, the severity of which is graded according to the scale by Namba, et al.(48)

Namba grade	Clinical presentation
Namba 1 or Latent	• No clinical manifestations • Assessment of severity is by measurement of serum cholinesterase level which will be inhibited by 10-50%
Namba II or Mild	• The patient is able to walk but complains of dizziness, headache, nausea and vomiting, numbness of extremities, excessive sweating and salivation, abdominal cramps, tightness in chest or diarrhoea • Serum cholinesterase level is 20-50% of normal
Namba III or Moderate	• The patient is unable to walk and there is difficulty talking, generalised weakness, muscular fasciculation, and miosis. • Cholinesterase level is 10-20% of normal
Namba IV or Severe	• Unconsciousness, loss of pupil reflex to light and marked miosis, flaccid paralysis, muscular fasciculation, secretions from the mouth, moist rales in the lungs and nose, respiratory difficulty and cyanosis. • Serum Cholinesterase levels < 10% of normal.

2. **Intermediate syndrome:** Patients may develop neuromuscular junction failure after the cholinergic crisis ends and may have sudden respiratory arrest. Intermediate syndrome was defined during a prior study in our institution as proximal muscle weakness of grade 3 or less with extra-ocular, neck and respiratory muscles weakness developing after 72 hours of consumption of the organophosphorus compound, which may or may not require mechanical ventilation.(49) Several mechanisms have been postulated for the development of intermediate syndrome including neuromuscular junction dysfunction(50), late release of organophosphorus compounds from adipose tissue resulting in delayed paralysis(51) and lack of early oxime therapy resulting in persistent nicotinic effects.(52,53) Other postulates were oxidative stress-induced myopathy, desensitisation or down regulation of acetylcholine receptors and muscle necrosis.(54) Methylparathion and monocrotophos were found to have high incidence of intermediate syndrome in a study conducted in India.(53) The usual presentation of intermediate syndrome is respiratory muscle weakness and treatment is mainly supportive till recovery which usually occurs between 3 to 12 days. During this period of intermediate syndrome, patients may develop altered consciousness manifesting the clinical entity known as delayed organophosphate encephalopathy (DOPE) or "CNS intermediate" in which patients have small miosed pupils.(55)
3. **Delayed manifestations:** Patients with OP poisoning can have delayed manifestations such as peripheral neuropathy and neuropsychiatric manifestations in form of behavioural changes.

MANAGEMENT OF ORGANOPHOSPHORUS POISONING

GENERAL MEASURES:

Early therapy for organophosphate poisoning is known to be life-saving. Supportive management including maintenance of airway, breathing and circulation and decontamination are imperative parts of care.(56) Information on the type of poison must also be obtained to further guide management. Gastric lavage may be effective if done early after ingestion of the compound. Although there are no randomised controlled trials evaluating its benefit in OP poisoning, it has theoretical benefit and should be considered in all patients early in the course of admission after initial resuscitation measures.(57) Activated charcoal has shown to reduce the in-vitro adsorption of organophosphorus compounds(58), but this result was not replicated in-vivo in a study done in Sri-Lanka where no benefit was found.(59) This discordance might be attributed to faster absorption of the compound into the blood and other factors such as large dose or late charcoal administration. At present, there is no evidence for administering charcoal in these patients.(3)

ATROPINE:

The mainstay of specific therapy for OP poisoning remains anticholinergic agents with clinical data showing the efficacy of atropine in preventing respiratory failure. Atropine blocks the effects of organophosphorus compounds on muscarinic sites. Atropine is given in incremental doubling doses until the chest is clear and to maintain a heart rate of more than 80 beats per minute followed by an infusion.(60)

ROLE OF OXIMES IN ORGANOPHOSPHORUS POISONING

Oximes show benefit in-vitro, in animal models and in accidental poisoning. The first trial showing the efficacy of oximes in organophosphorus poisoning was done in the 1950s on workers exposed to the compound.(61) Over the years, the results of various trials done to determine the efficacy of oximes in organophosphorus poisoning have been inconclusive. Few clinical trials have shown efficacy of oximes in the setting of mega-dose OP poisoning. Hence their role as a specific therapy for organophosphate poisoning remains controversial.

WHO recommends that oximes may be used for the treatment of organophosphorus poisoning.(56) The previously used dose of pralidoxime of 1g of loading followed by repeat doses hourly in severe poisoning was replaced as it had been shown to produce sub-therapeutic levels of oxime. The alternative recommendation was 2g of loading followed by 500mg/hour infusion (30mg/kg bolus which is followed by 8mg/kg/hour continuous infusion) to achieve pralidoxime levels in blood above 4mg/L.(62,63) Obidoxime dose recommended was 3mg/kg as slow intravenous infusion followed by 0.4mg/kg/hour. An uncontrolled study showed the benefit of pralidoxime in OP poisoning.(47) However, a retrospective study detailed the clinical outcomes in Sri Lanka before and after pralidoxime became available. It found that there was no significant difference when pralidoxime was added to atropine therapy.(64) Other retrospective trials also did not find any statistically significant benefit with pralidoxime therapy. There was no added benefit of oximes over the standard therapy with atropine along with adequate general measures, especially respiratory support.(64–66) However, one retrospective study noted the presence of

significant reactivability of the acetylcholinesterase enzyme associated with pralidoxime therapy although there was no significant clinical effect of the same.(66)

Study	Study Design (No. of patients)	Interventions	Results
Duval (1991)	Retrospective comparison (N = 62)	Pralidoxime (1200 mg/24 hr) vs standard treatment	No statistical difference in the risk of death or the need for ventilation between the treatment groups
De Silva (1992)	Historical comparison (N = 45)	Pralidoxime (4 g over first 24 hr then 1 g/day) vs historical control	No statistical difference in the risk of death, the need for ventilation or the rate of intermediate syndrome between the treatment groups
Abdollahi (1995)	Retrospective comparison (N = 34)	Pralidoxime (600-800 mg every 4-8 hrs, based on patient condition) vs standard treatment	No statistical difference in the risk of death or the need for ventilation between the treatment groups
Samuel (1995)	RCT (N = 72)	High dose pralidoxime (12 g reducing infusion over 4 days) vs low dose pralidoxime (1 g bolus)	High dose pralidoxime was associated with a significantly higher risk of death, need for ventilation and rate of intermediate syndrome
Cherian (1997)	RCT (N = 110)	Pralidoxime (12 g infusion over 3 days) vs placebo (and standard care)	Pralidoxime was associated with a significantly higher risk of death, need for ventilation and rate of intermediate syndrome
Balali-Mood (1998)	Prospective comparison (N = 72)	Pralidoxime (14 ± 7.4 g), obidoxime (60.6 ± 24.3 g) vs standard treatment	Pralidoxime and obidoxime were associated with more respiratory complications. No deaths were observed in the pralidoxime arm. Deaths were observed in the obidoxime and standard care arms
Sungur (2001)	Retrospective comparison (N = 47)	Pralidoxime (3.5 ± 3.0 g) vs standard treatment	No statistical difference in the risk of death between the treatment groups
Cherian (2005)	RCT (N = 21)	Pralidoxime (12 g/day (severe) or 4 g/day (moderate) over 3 days) vs placebo (and standard care)	No statistical difference in the risk of death or the need for ventilation between the treatment groups
Chugh (2005)	Prospective comparison (N = 30)	Pralidoxime (1 g/6 hrs) vs standard treatment	No statistical difference in the risk of death or the need for ventilation between the treatment groups

Figure 7: Studies which were included in systematic reviews of oximes in organophosphorus poisoning (62)

The above figure 7 summarises the various trials, both retrospective and randomised, that have studied oxime therapy for poisoning due to organophosphorus compounds. Despite all the above trials not showing any benefit of oxime therapy, the results may have been confounded by other factors such as limitations of methodology, inadequate reporting and small sample sizes for all the studies. Many of the above studies did not follow the WHO guidelines for dosing of pralidoxime. Some of the studies used bolus doses of oximes instead of infusions and in some, there was no bolus dose given prior to start of infusion. Two studies have detailed the superiority of the WHO regimen consisting of 2g stat dose of pralidoxime followed by continuous infusion with lower rates of intermediate syndrome, requirement for mechanical ventilation and lower total atropine dose required compared to the repetitive bolus dose injection.(67,68)

Several systematic reviews have studied the effectiveness of pralidoxime with the same results as the individual trials with no significant statistical difference in mortality or other complications as shown in figure 8. In the review by *Eddleston* et al, it was further found that there was no benefit for oximes across different sub-groups analyses including dimethyl and diethyl class of OPs, those presenting less than and more than 4 hours after admission and in those presenting with differing levels of severity.(69)

Systematic review (year)	Results/conclusions
Eddleston (2002)	• Descriptive analysis of Abdollahi (1995), Samuel (1995), Cherian (1997) and Dadan (1999) **Conclusion**: The current evidence is insufficient to indicate whether pralidoxime is harmful or beneficial in the management of acute organophosphorus pesticide poisoning
Buckley (2005)	• Descriptive analysis of Samuel (1995) and Cherian (1997) **Conclusion**: The current evidence is insufficient to indicate whether oximes are harmful or beneficial in the management of acute organophosphorus pesticide poisoning
Bairy (2006)	• Descriptive analysis of De Silva (1992), Abdollahi (1995), Samuel (1995), Cherian (1997), Balali-Mood (1998), Cherian (2005), Chugh (2005), and Dadan (1999) **Conclusion**: The clinical benefits of oximes in OP poisoning remains unclear
Peter (2006)	• Meta-analysis of Duval (1991), De Silva (1992), Abdollahi (1995), Cherian (1997), Balali-Mood (1998), Sungur (2001) and Cherian (2005) ○ Oxime therapy was not associated with a statistically significant difference in mortality (RD 0.09, 95% CI -0.08, 0.27); need for mechanical ventilation (RD 0.16, 95% CI -0.07, 0.38), incidence of intermediate syndrome (RD 0.16, 95% CI -0.12, 0.45) compared to standard care. ○ Oxime therapy was associated with a statistically significant increase in the need for intensive care (RD 0.19, 95% CI 0.01, 0.36) compared to standard care **Conclusion**: Based on the current available data on human organophosphate poisoning, oxime therapy was associated with either a null effect or possible harm
Rahimi (2006)	• Meta-analysis of De Silva (1992), Abdollahi (1995), Cherian (1997), Balali-Mood (1998), Sungur (2001) and Cherian (2005) ○ Oxime therapy was associated with a statistically significant increase in mortality (RR 2.17, 95% CI 1.34, 3.51); need for mechanical ventilation (RR 1.53, 95% CI 1.16, 2.02), incidence of intermediate syndrome (RR 1.57, 95% CI 1.11, 2.11) compared to standard care. **Conclusion**: Oximes are not effective in the management of organophosphate-poisoned patients and can worsen the patient's clinical situation

Figure 8: Systematic reviews assessing the effectiveness of pralidoxime in organophosphorus poisoning (60)

There may be several factors which have contributed to this apparent lack of in-vivo benefit including poor affinity of the oxime for the particular complex of OP with AChE, low dose or inadequate duration of treatment, time interval between

poison ingestion and oxime administration, reinhibition of the reactivated enzyme due to the persistence of the poison in the patient and variable ageing times of different compounds. In view of the above factors and the additional confounding factors in each study, it was concluded that the above trials were insufficient to reach a conclusion regarding the usefulness of oxime therapy in OP poisoning.(4)

TRIALS AT CHRISTIAN MEDICAL COLLEGE, VELLORE

Two Randomised Control Trials done in our institution did not show any benefit from pralidoxime therapy. The first trial included 72 patients and compared low-dose(1g bolus) against high-dose pralidoxime (12g as infusion over 4 days) and showed worse outcomes in the second group with higher rates of ventilator requirement and intermediate syndrome, although these event rates were not statistically significant.(70) However, a sub-group analysis showed lower incidence of intermediate syndrome in those whom pralidoxime was administered within 12 hours of ingestion indicating benefit for early therapy. The second trial was placebo-controlled with 110 patients getting high-dose pralidoxime (12g over 3 days). This also showed higher mortality rates in the high-dose group as well as higher rates of ventilation.(71) A systematic review of four trials including the above two trials questioned whether therapeutic levels of pralidoxime were reached as bolus dose was not given and the current WHO-recommended dose was not implemented during the time period of both the studies.(4) The ‘harm’ that occurred in the second study may be related to a selection bias as the sicker patients may have been in the high-dose group.

CONTROVERSY REGARDING OXIME THERAPY

The WHO recommendation of pralidoxime and obidoxime dose was based on an in-vitro and animal study which determined the dose of pralidoxime required to reach therapeutic levels rapidly and to sustain a high concentration.(72) This dose may, however, represent an under dosing in patients with mega-dose OP poisoning due to the variable kinetics displayed by the different compounds. The complete therapeutic potential of pralidoxime may not be exploited at this dose. In cases of severe OP poisoning with large doses, there may be rapid re-inhibition of the reactivated enzyme especially in the initial few days after poisoning which may render pralidoxime ineffective particularly when it is administered at lower doses.(73)

A Cochrane review also showed insufficient evidence for the use of oximes.(74) It, however, included only one RCT with the WHO recommended dose consisting of 30mg/kg bolus followed by 8mg/kg/hour infusion which was conducted in Sri Lanka and randomised 235 patients to either receive pralidoxime or saline placebo. The primary outcome of mortality was not significantly different between the two groups. The levels of pralidoxime were also measured and there was no difference in levels between survivors and those who died. Red cell AChE levels were measured and there was in-vitro reactivation of enzymes inhibited by OPs belonging to diethyl class but not dimethyl class of compounds.(69)

Due to these multiple in-vivo studies which have failed to show sufficient benefit from oxime therapy, their use had been discontinued in our institution. The Cochrane analysis and systematic reviews have shown that the results from these trials may have been affected by several factors including timing of administration of

oximes, the dose of pralidoxime and type of organophosphorus compound. Organophosphate poisoning is a heterogeneous clinical condition, both in the variety of compounds, amount of poison ingested, the variability of time to presentation, differences in decontamination, absorption and metabolism.

It is possible that a sub-group of patients with organophosphate poisoning may benefit from oxime therapy. *Eddleston* et al postulated that there may have been a benefit from oximes in a sub-group of patients which may have been masked by the ineffectiveness in the majority. It was suggested that there may be a role for selective administration of oximes for certain clinical settings of organophosphorus poisoning.(42,69)

PLASMA BUTYRYLCHOLINESTERASE (BChE) LEVELS

The above postulate of selective administration of oximes led to several studies to determine optimal administration of oximes in certain clinical setting with only selected organophosphorus compounds. In-vitro trials have attempted to determine the reactivation of the cholinesterase enzymes after application of oximes in the laboratory. It was attempted to apply these results later to clinical care of the patients with organophosphorus poisoning. Most in-vitro trials on oxime efficacy have been conducted with plasma butyrylcholinesterase (BChE). Reactivation potential of butyrlcholinesterase and acetylcholinesterase is defined as the difference between oxime-reactivated enzyme and unreactivated enzyme activity. This reactivation

potential and subsequent clinical consequences of the same have not been sufficiently analysed.

A study done in our institution showed that plasma BChE activity correlated with the severity of OP poisoning and that reactivation potentials were lower for patients who had developed intermediate syndrome and those with greater severity. However, there was no increase in BChE levels in those who had received oximes prior to admission.(49) As expected the BChE levels showed a decremental response as the time after poison ingestion increased. This study showed that the BChE values may correlate well with the severity of poisoning in clinical settings and the temporal profile may correlate with the clinical course. However, the pathophysiology of OP poisoning is based on the inhibition of Acetylcholinesterase (AChE) in the synapses. Plasma BChE is being used as an indirect marker of enzyme activity in place of AChE and is not directly involved in the pathogenesis of toxicity in organophosphorus poisoning. A study showed that the reactivation of BChE by pralidoxime in Sri Lankan patients with OP poisoning was variable and was not sustained.(75) Pralidoxime reactivated diethyl compounds at a 2g bolus dose but not with the 1g bolus dose and this reactivation was poorly sustained. There was no improvement in enzyme activity in dimethyl compound poisoning. There was significant variability in individual responses of cholinesterases when given pralidoxime. Therefore plasma cholinesterase levels may not be sufficient to assess the efficacy of oximes. Its usefulness is also determined by the type of OP compound as a level of <600mU/ml on admission was highly sensitive for chlorpyrifos but more specific for dimethoate poisoning.(76)

Questions have arisen about the appropriateness of butyrylcholinesterase activity as a test of oxime efficacy in view of the conflicting findings of several studies determining the correlation of BChE and its reactivation with the synaptic AChE levels. Pralidoxime at doses of 100mcM and Obidoxime at dose of 175mcg/ml have been shown to reactivate BChE which had been inactivated by parathion, paroxon and chlorpyrifos.(77,78) However, two more in-vitro studies showed contradictory findings and found no reactivation of BChE with either pralidoxime or obidoxime in parathion and methylparathion poisoning.(79,80) This led to the recommendation that BChE was an inappropriate test for studying the efficacy of oximes in organophosphorus poisoning.(79) The main utility of BChE lies in diagnosis of OP poisoning in doubtful cases and to monitor OP elimination from the body as the liver synthesises BChE continuously in contrast to AChE which requires erythropoiesis for regeneration. Therefore, once the inhibitory organophosphorus compound is eliminated from the bloodstream, there should be rapid rise in butyrylcholinesterase levels which would signify the culmination of the cholinergic crisis.(81)

ERYTHROCYTE ACETYLCHOLINESTERASE (AChE) LEVELS

It was suggested that measurement of RBC AChE would be a better marker for determining the severity of OP poisoning and to further study the efficacy of oximes than BChE. AChE in mammals is coded by a single gene and therefore, the structure of the RBC-AChE should be similar to that found in the synapses. RBC-AChE is, thus used as a surrogate marker for the activity of the synaptic enzyme and is expected to

react similarly with the organophosphorus compound.(82) RBC-AChE has been shown to correlate better with neuromuscular transmission. A level of more than 30% was found to correlate with normal muscle function and precludes the use of atropine whereas a level of less than 10% correlated with severe neuromuscular transmission reflected by a decremental response.(83) In a dynamic in-vitro muscle model, the erythrocyte AChE level was found to correlate well with the muscle membrane AChE reflecting that RBC-AChE is a good parameter for the studying the mechanisms of Organophosphorus-related inhibition, ageing kinetics as well as reactivation kinetics both spontaneously and with oxime therapy. These in-vitro results were also applied to parathion poisoned patients for study and there was favourable correlation between the clinical data and the laboratory findings.(84) In patients with parathion poisoning, the cholinergic signs resolved after the RBC-AChE levels rose to more than 20%.(46,85) However, RBC-AChE is not a perfect test and there are certain disadvantages to it use in clinical practice and research settings. RBC-AChE levels recover less rapidly than muscle function recovery and may not be a good marker of improvement. This is because acetylcholine is reproduced only be production of new RBCs and this regeneration is a slow process occurring at a rate of 1% per day of total AChE level. It takes approximately 5 weeks for the AChE levels to return to normal after OP poison consumption.(86) The assays are also more cumbersome as they have to be done on whole blood which is immediately cooled after dilution and inhibitor must be added to block BChE activity. This should be done to block the on-going reactions between the organophosphorus compound, acetylcholinesterase and oximes which would continue if the sample was left even for few minutes at room

temperature. They are also sensitive to the level of oxime, the substrate and pH.(3) To be reliable, the sample must be collected and cooled and diluted rapidly to get accurate results. In many cases, it was also found that the levels of AChE did not uniformly conform with the clinical picture which may be related to the variable distribution and excess AChE distributed across the different tissues of the body.(87)

TRIPLE CHOLINESTERASE TEST

Certain studies have suggested the apparent lack of efficacy of oxime therapy may be due to sub-therapeutic doses of oximes and have propounded that oxime therapy must be continued as long as there is reactivation potential in the inhibited acetylcholinesterase enzyme. This led to the concept of determination of reactivation potential of the enzyme to guide oxime therapy. Many of the above clinical studies showing ineffectiveness of oxime therapy were limited by the under dosing of oximes in the trial. Based on theoretical considerations and in-vitro and clinical findings, it was concluded that oximes must be administered as long as there was reactivability of the acetylcholinesterase enzyme and until definitive and complete clinical improvement.(7,73) *Thiermann* et al studied the use of such a regimen of oxime therapy with objective parameters consisting of the following tests: RBC-AChE, reactivability of RBC-AChE ex vivo, plasma ChE activity, AChE inhibiting compounds and concentrations of obidoxime and atropine in patient's plasma. A bolus dose of obidoxime followed by infusion was administered and levels of 10-20mM were attained in accordance to the previous kinetic studies.(6,7) Obidoxime therapy was effective when administered early especially in parathion poisoning. Enzyme

reactivation was almost complete when obidoxime was administered within one hour after consumption of the compound. It was not effective for patients with oxydemetonmethyl poisoning. This study, however, was limited by a small sample size as it included only 5 patients.(7) The group involved in the above study postulated that the above advanced laboratory monitoring system determining the cholinesterase status of the patient can be used to effectively predict and follow-up the clinical course in patients with organophosphorus poisoning and also guide the rational use of oxime therapy. Based on the data from these tests, oxime therapy could be individualised on a case-to-case basis.(82)

The triple cholinesterase test is a laboratory system which was devised to determine the cholinesterase status of the patient and consists of the following battery of investigations:

1. **Plasma Butyrylcholinesterase**
2. **RBC Acetylcholinesterase**
3. **Reactivation potential of RBC-AChE ex-vivo**
    - Defined as the difference between the enzyme activity after reactivation by oximes and the enzyme activity prior to oxime administration
    - Can be studied by measuring RBC-AChE levels after incubating whole blood with obidoxime
4. **Inhibitory potential of patient's plasma**
    - Reflects the presence of poison in the plasma of the patient but does not involve identification of the organophosphorus compound
    - Can be determined by incubating the patient's plasma with standardised RBC-AChE derived from a healthy donor

This cholinesterase status testing was done in animal experiments on pigs poisoned with sarin and oximes were administered according to this status. A rapid rise in RBC-AChE activity was noted with prompt clinical resolution of symptoms and signs. Neuromuscular transmission was also studied to confirm recovery as an objective clinical parameter especially when the AChE was completely aged and it was difficult to determine the end of the cholinergic crisis. This study suggested that oximes should be given as early as possible in all patients with OP poisoning to be effective and their effects can be assessed by the triple cholinesterase test status. It affirmed the findings of the previous study and recommended continued oxime administration till reactivation was possible and there was absence of inhibitory substance in the patient's plasma.(8,88)

The assessment of cholinesterase status as a guide to oxime therapy has not been done on a large number of patients with organophosphorus poisoning. As earlier suggested, there may be a sub-group of patients who may benefit from oxime therapy. The diagnostic challenge is to identify this group of patients. By study the temporal profile of the triple cholinesterase status in different clinical settings and correlating with the patient and organophosphorus compound characteristics, it may be possible to identify the clinical characteristics of the sub-group of patients with good enzyme reactivability with oximes. Identifying such a group of patients, may allow for a tightly controlled clinical trial to evaluate the benefit of oximes in patients who may potentially benefit from this treatment. We attempt to validate the triple cholinesterase test for point-of-care testing to determine the characteristics of the sub-group of patients in whom oxime therapy could potentially be useful.

JUSTIFICATION

The study consists of two parts as follows:

A. To study the role of triple cholinesterase test in the management of Organophosphorus (OP) poisoning

Organophosphorus insecticides are familiar household products which are the most common suicidal agents used in Asian countries. While early treatment is known to be life-saving, specific therapy for organophosphate poisoning still revolves around anticholinergic agents which however cannot revive the acetylcholinesterase enzyme that is inhibited by the organophosphorus at the synapses. Oximes such as Pralidoxime and Obidoxime have been shown to reactivate the cholinesterase enzyme which had been phosphorylated by binding to the OP molecule with obidoxime being more potent than the other oximes. However, their role in routine management of OP poisoning remains controversial. Several in-vitro studies have shown their potency in reactivating the cholinesterase enzyme. However, the same results have not been replicated in many clinical studies. Furthermore, their ability to reactivate the enzyme depends on several factors that affect the aging of the OP-enzyme compound such as timing of administration, the type of organophosphorus compound, the quantity of the compound, pH and temperature. Due to these reasons, oxime therapy has not been universally accepted as part of routine management. Studies done in recent times have suggested that there may be certain sub-groups of patients in whom oximes would be beneficial. A new concept of determination of cholinesterase status to guide oxime

therapy has also been put forth wherein the oxime is administered as long as there is reactivation of the AChE in-vitro. This study aims to answer the following questions:

1. What is the in-vitro potential of obidoxime to reactivate OP inhibited cholinesterase enzymes?

2. Can a battery of tests including RBC acetylcholinesterase (AChE), Plasma butyrylcholinesterase (BChE) and enzyme reactivating potential of obidoxime in-vitro be applied at point-of-care for patients with OP poisoning to determine their suitability for oxime therapy?

3. In what clinical situations, would oxime therapy be useful?

B. Changing trends in the profile of poisoning presenting to a tertiary care centre in South India.

During another toxicological study in the same centre on plant poisoning, it was found that there was sharp decline in the number of plant poisoning cases. By analysing the poisoning database of this hospital, it would be possible to determine the changing profile of adult poisoning presenting to our hospital in relation to the major classes of poisoning, their epidemiology and the outcomes. This would help in toxicovigilance activities at a regional level.

METHODOLOGY – (A) TRIPLE CHOLINESTERASE TEST IN ORGANOPHOSPHORUS POISONING:

SAMPLE AND SETTING

This study was conducted in the period between April 2015 and August 2015 in Christian Medical College, Vellore. All patients who presented to adult emergency department with organophosphorus poisoning fulfilling the inclusion criteria and willing to participate in the study were included in the study. The procedures of the study were explained in detail to the participants and their close relatives and they were provided with an information sheet about the study (Annexure 1). Participants were included only after obtaining written consent either from the patient directly or from their close relative if the participant was otherwise indisposed (Annexure 2). Consent was obtained in the participants' native language. In case of consent obtained from relative, re-consent was obtained from the participant after recovery. For participants between the ages of 15 and 18 years, consent was obtained from the legal guardian and assent was taken from the participant.

STUDY DESIGN

This is a prospective cohort study done as pilot study in patients presenting with organophosphorus poisoning to evaluate the triple cholinesterase test.

SAMPLE SIZE

There are no studies that have correlated cholinesterase studies to the clinical profile. Hence it was not possible to calculate the sample size for the clinical study. So after discussion with our collaborators, we decided on sample size of 30 for a pilot

study. Based on previous studies we expect an adequate distribution of clinical factors that may determine oxime reactivability: 50% dimethyl and diethyl OP compounds; 20% with prior oxime use; 40% who will present in less than 6 hours after ingestion and 60% with severe poisoning and 40% with mild and moderate poisoning.

PARTICIPANTS

All patients presenting to adult emergency department in CMC, Vellore within 30 hours of ingestion of organophosphorus compound were included in the study. The following were included as the case definitions for organophosphorus poisoning:

1. Patients who present with history of pesticide poisoning with an identified OP compound.
2. Patients who present with history of pesticide poisoning with typical toxidrome of organophosphate poisoning, and low BChE levels (<3000 U/L).
3. Patients who present without a history of pesticide poisoning but with typical toxidrome of OP poisoning and low BChE levels (<3000 U/L)

Those presenting after 30 hours of OP consumption and those less than 15 years of age were excluded from the study. Persons who had consumed mixed compounds of organophosphorus with pyrethroids were not excluded. Pregnant women and children between 15 and 18 years of age were also included as OP poisoning can occur in any person and as this is an observational study with no harm expected to the patients.

MEASUREMENTS – DATA AND SAMPLE COLLECTION

Data collection was done by the principal investigator at patient presentation to CMC, Vellore followed by serial assessment 12 hours later and subsequently once

daily between 6-8 PM till discharge. The clinical data was documented in the patient proforma (Annexure 3) by the principal investigator. The following details were noted specifically

1. **Compound characteristics** – class, quantity and combination with pyrethroids
2. **Time to presentation to CMC, Vellore**
3. **Treatment elsewhere** – Gastric lavage, Atropine and Pralidoxime received including the doses
4. **Severity at presentation** (by Namba scale)
5. **Outcomes** – Dose of atropine required and duration, Mechanical ventilation and duration, Intermediate syndrome and duration, Need for ICU admission and duration

Samples for triple cholinesterase test were collected at admission not later than 30 hours after OP consumption followed by repeat sample 12 hours later and subsequently once daily at 12 PM for 5 days from poison consumption. The protocol for data and sample collection has been detailed in Annexure 4.

DETERMINATION OF CHOLINESTERASE STATUS

All patients had the following investigations done on the samples collected as mentioned above:

1. RBC-AChE activity
2. Plasma BChE activity
3. RBC-AChE reactivation potential with obidoxime ex-vivo
4. Inhibitor activity of patient's plasma on control RBC-AChE

Reference standard used in our lab is measurement of plasma BChE and RBC-AChE. We use modified Ellman assay for detection of cholinesterase levels in our lab. After recruitment into the study, venous blood samples were collected from the patient and 2 ml of blood was added to a purple EDTA tube. For RBC AChE analysis, from the collected blood samples, 200 microlitres of blood was measured using a pipette and diluted (20 times) in 4 ml of cold normal saline (at 40 degree C) to prevent hemolysis, which was then well mixed and stored in -20 degree Celsius in the refrigerator within 5 minutes. The blood samples were sent to laboratory in batches at the end of 5 days for each patient. The remaining sample in the EDTA tube was then sent for BChE analysis immediately to the lab. Both the assays used the same principle measured through an automated assay. This method measures hydrolysis of the substrate acetylthiocholine (ATCh) by either BChE or AChE to yield acetate and thiocholine. The latter product reacts with 5, 5'-dithiobis-2-nitrobenzoic acid to produce a yellow-coloured acid anion (5-thio-thionitrobenzoic acid) that can be measured with a spectrophotometer. The results were expressed as micromoles per minute per milliliter (μmol/min/mL) and adjusted for haemoglobin absorbance.

STANDARD OPERATING PROTOCOL OF TRIPLE CHOLINESTERASE TEST

The determination of the cholinesterase status was done according to the standard operating protocol used by *Thierman* and *Worek et al* in their previous study and was discussed with them. All laboratory measurements were based on the recommendations of *Eyer* et al and standardised for our laboratory analysers.(88,89) The complete details of the test and measurements are detailed in Annexure 5.

DATA ANALYSIS AND STATISTICAL METHODS

STATISTICAL METHODS

Out of 36 patients screened for OP poisoning, 6 were excluded, 4 as they presented after 30 hours and 2 as they were later found to be due to carbamate poisoning. 30 patients were included for the final analysis. The primary outcome involved the comparison of temporal profile of RBC acetylcholinesterase, reactivability and plasma inhibitory activity on AChE (mean absolute value on Day 1, day 3 and day 5) in the following groups:

1. Severity of poisoning (mild vs. moderate/severe)
2. Dimethyl vs. Diethyl compounds
3. Mechanical ventilation vs. no mechanical ventilation
4. Intermediate syndrome vs. no intermediate syndrome
5. Prior exposure to oximes vs. oxime-naïve patients

The secondary outcome was the description of clinical characteristics of (1) Patients who have RBC AChE reactivability at admission and for the first 3 days and (2) Patients who have persisting plasma cholinesterase inhibitory activity.

In view of the small number of patients included in this pilot study, non-parametric statistical tests such as Mann-Whitney U test and Kruskal-Wallis tests were used to analyse the data and to determine the significance level of the differences among the groups.

METHODOLOGY – (B) STUDY OF POISONING PROFILE USING THE POISON DATABASE:

Medicine unit 1 in CMC, Vellore has been functioning as a poison control centre and prospectively collecting the poisoning data base from 2009. *(AC approval for the establishment of the Poison control centre and development of the CMC poison database – Min. No. 5-a(4):2-15 dt. 19.02.2015)* We have reviewed over 2300 poisoning proformas collected by the Poison centre between 2009-2014 and obtained data relevant for this study based on data abstraction form (Annexure 6). We verified data where required from inpatient discharge summaries and outpatient scanned charts from the electronic record system. The following details of interest for the study were collected from the poisoning proforma:

1. Demographic details – age, sex, occupation

2. Mode of poisoning – Accidental /Deliberate self-harm

3. Poison details (Chemical name and Class of compound)

4. Status at admission – Casualty/Ward admission/ICU admission

5. Outcome – Dead/Alive/Discharge against medical advice

FUNDING AND APPROVAL

SOURCE OF FUNDING

A FLUID research grant was approved from the institution for the purpose of this study. The funds were used for the cholinesterase status assays and for procurement of the reagents and obidoxime.

INSTITUTIONAL RESEARCH BOARD APPROVAL AND ETHICAL CONSIDERATIONS

The research proposal for the triple cholinesterase test was discussed by the Institutional Review Board in 2015 and approval was obtained [IRB Min. No. 9463 dated 05.06.2015]. There were no ethical issues related to this study. Institutional review board approval was obtained for the procedures. The study on the poison database was also discussed by the IRB and approval was obtained [IRB Min. No. 9507 dated 24.06.2015].

RESULTS – (A) TRIPLE CHOLINESTERASE TEST IN ORGANOPHOSPHORUS POISONING

DEMOGRAPHIC CHARACTERISTICS

30 patients, 15 years of age or older, admitted to adult emergency department with organophosphorus poisoning within 30 hours of consumption were included in the study. The mean age (±S.D) was 28.4 years (±11.82 years) with a range of 15-64 years. These patients were recruited between April 2015 and August 2015. Out of 30 patients, 14 were male and 16 were female. Of the female patients, one patient was pregnant and in the 2nd trimester of pregnancy. 16 out of 30 (53.3%) patients were uneducated and 24 (80%) were occupied in unskilled labour. (Table 1)

COMPOUND CHARACTERISTICS

Out of 30 patients, the compound was not identified in 4 patients and the case ascertainment criteria used was patient presenting with history of pesticide poisoning with typical toxidrome of OP poisoning and with low BChE. Among the 26 cases, in 4 cases, the leaflet was brought and in the remaining 22 cases, the bottle of pesticide was brought by the patient's relatives.

The most common compound used was monocrotophos accounting for 5 cases (16.7%). There were 4 cases each of dimethoate, profenofos and triazophos. The other compounds accounted for only one or two cases. 6 of the compounds had pyrethroids mixed with the OP, cypermethrin in 4 cases and deltamethrin in 2 cases. The most

common combined formulation was with chlorpyrifos and triazophos. The OP compounds were further categorised based on their chemical nature and dimethyl compounds accounted for 12(40%) of all OP poisonings and diethyl compounds for 10(33.3%). The S-alkyl compound used was profenofos accounting for 4(13%) of cases. (Table 1)

Table 1: Patient and compound characteristics of OP poisoning cases

Baseline characteristics	Details (n=30)
Identified OP	26
Age (years) ± S.D.	28.4 ± 11.8
Male, n(%)	46.7% (n=14)
Compounds (in number of patients)	Monocrotophos – 5 Dimethoate – 4 Profenofos – 4 Triazophos – 4 Chlorpyrifos – 2 Quinalphos – 2 Phorate – 2 Dichlorvos, Methylparathion, Malathion – 1 each
Compound type	Dimethyl – 40% (n=12) Diethyl – 33% (n=10) S-Alkyl – 13% (n=4)
Pyrethroid combination with OP	20% (n=6) 4 – cypermethrin 2 – deltamethrin

TREATMENT ELSEWHERE PRIOR TO PRESENTATION

Of the 30 patients, 20 had received treatment elsewhere prior to presentation. The mean time interval between consumption and first medical contact was 2.05 hours

(± S.D 2.01). The time interval between consumption and presentation to CMC, Vellore was 6.6 hours (± S.D 5.84) ranging from 1 hour to 29 hours. 18 patients had received gastric lavage elsewhere prior to admission. 17 received atropine prior to presentation with doses ranging from 2 ampules to 20 ampules and with dose given being unknown in 10 cases. Pralidoxime had been given in 6 patients. In 2 of these patients, the dose given had been documented to be stat doses of 2g and 0.5g and among the others, the dose of PAM given was unknown. 4 patients had been intubated before admission here.

TOXIDROME FEATURES AT PRESENTATION

The clinical symptoms at presentation are summarised in the table 2 below

Table 2: Toxidrome features present at admission in the OP poisoning patients

Toxidrome present	Number of patients(%)
Salivation	27 (90%)
Lacrimation	6 (20%)
Diaphoresis	9 (30%)
Urination	9 (30%)
Defecation	3 (10%)
Vomiting	27 (90%)
Seizures	3 (10%)
Breathlessness	8 (26.7%)
Altered sensorium	9 (30%)

The clinical signs at presentation and the overall severity according to the Namba scale are represented in the table 3 below

Table 3: Clinical signs present at admission in the OP poisoning patients

Clinical signs	Number of patients with % (or Mean ± S.D)
GCS, n(%)	
15/15	16 (53.3%)
<10/15	7 (23.5%)
Pupil size, n(%)	
Pinpoint	11 (36.7%)
Dilated	3 (10%)
Normal (2-5mm)	16 (53.3%)
Heart rate, mean (± S.D)	102.53 (± 19.48) beats per minute
Blood pressure, mean (± S.D)	110 (±16) / 70 (±10) mm Hg
Respiratory rate, mean (± S.D)	
Mean RR	23 (±6.5) per minute
Intubated	3 (10%)
Gasping	3 (10%)
O2 saturation, mean (± S.D)	93 (± 8.8) %
Blood sugar, mean (± S.D)	174.3 (± 56.6) mg%
Fasciculations present, n(%)	8 (26.7%)
Single breath count, mean (± S.D)	
Mean	13 (±12) counts
Could not be assessed	8 (26.7%)
Neck holding time , mean (± S.D)	
Mean	16 (± 18) seconds
Could not be assessed	6 (20%)
Severity (Namba scale), n(%)	
Latent	0
Mild	11 (36.7%)
Moderate	7 (23.3%)
Severe	12 (40%)

CLINICAL OUTCOMES

The overall clinical outcomes including intermediate syndrome, ICU admission, mechanical ventilation and dose and duration of atropine are summarised in the table 3 below.

Table 4: Clinical outcomes of the OP poisoning patients

Clinical outcome	Number of patients with % (or Mean ± S.D)
Intermediate syndrome, n(%)	
Present	10 (30%)
Duration	7.7 (± 4.9) days
ICU admission, n(%)	
Present	17 (56.7%)
Duration	8 (± 5) days
Mechanical ventilation, n(%)	
Present	17 (56.7%)
Duration	6.9 (± 4.5) days
Tracheostomy required, n(%)	3 (10%)
Infective complications present, n(%)	10 (33.3%)
VAP	9 (30%)
Bacteraemia	2 (6.6%)
Low GCS, n(%)	11 (36.6%)
Atropine	
Required, n(%)	28 (93.3%)
Dose, mean (± S.D.)	243 (± 406) mg
Duration, mean (± S.D.)	3 (± 1.3) days
Hospitalisation duration, mean (± S.D.)	9.3 (± 7.1) days
Death, n(%)	3 (10%)
(Causes of death)	(2 – Infection, 1 – Ventilator and airway problems)

CALCULATION OF AChE LEVELS

AChE level was calculated based on the Ellman method as described in the methodology and expressed as µmol/min/µmol Hb. AChE value was done on 20 controls and 18 patients who had consumed pesticides which were not OP compounds or carbamate compounds. The median of the AChE values of this group was used to calculate the normal level which was found to be 16.18 µmol/min/µmol Hb (IQR

12.74–22.71). All subsequent AChE calculations of the patients including the baseline (pre-oxime) and pots-oxime levels were expressed in their absolute values and also in terms of a percentage of the normal AChE level.

DEFINITION OF SIGNIFICANT REACTIVATION

Previous studies based on neuromuscular transmission in OP poisoning by *Thiermann* et al had shown that at a level of AChE less than 10% of normal, neuromuscular transmission was severely disturbed and this level was noted to indicate urgent need for mechanical ventilation. Levels above 30% did not have significant disturbance of transmission. Levels in between were associated with moderate poisoning.(8) Based on this study, significant reactivation was said to have occurred if the AChE, which was less than 30% at baseline, was reactivated to more than 30% after addition of obidoxime to the sample.

BASELINE AChE LEVEL AND CLINICAL CHARACTERISTICS

The 30 patients underwent serial blood sampling for 5 days or up to discharge. AChE, obidoxime induced reactivated AChE and inhibitor activity performed on serial samples. The patients were divided into 3 groups based on their baseline AChE levels (Table 5). The groups were classified based on studies on the baseline AChE.

1. **Group I – baseline AChE level < 10% of normal (5 patients)**
    - Severe inhibition of AChE; likely impairment of neuromuscular transmission
2. **Group II – baseline AChE level 10-30% of normal (13 patients)**

- Moderate inhibition of AChE; likely impairment of neuromuscular transmission

3. **Group III – baseline AChE level >30% of normal (12 patients)**

    - Mild inhibition of AChE; likely normal neuromuscular transmission

Table 5: Clinical characteristics of groups according to baseline AChE level at admission

	Group I – AChE <10% of normal n=5	Group II – AChE 10-30% of normal n=13	Group III – AChE >30% of normal n=12	p value
Lag time to presentation to CMC – hours (±SD)	11.22(10.68)	7.11(4.33)	4.13(3.88)	0.503
Prior oxime, n(%)	1(20%)	2(15.4%)	3(25%)	0.157
Compounds, n(%)				0.080
dimethyl	3(60%)	4(30.8%)	5(41.7%)	
diethyl	0	6(46.1%)	4(33.3%)	
Severity, n(%)				0.002
mild	0	3(23.1%)	8(66.6%)	
moderate	1(20%)	4(30.8%)	2(16.7%)	
severe	4(80%)	6(46.1%)	2(16.7%)	
Intermediate syndrome, n(%)	3(60%)	5(38.5%)	2(16.7%)	0.066
IS duration – days (±SD)	9.3(7.5)	8.2(4.2)	4.0(1.4)	1.00
Mechanical Ventilation, n(%)	5(100%)	8(61.5%)	4(33.3%)	0.010
Ventilation duration – days (±SD)	8.0(6.0)	7.5(4.1)	4.2(2.9)	0.724
Atropine dose (mg)	417.18	278.55	91.74	0.117
Atropine duration – days (±SD)	3.2(1.3)	3.3(1.5)	2.2(1.4)	0.924
Hospitalisation duration – days (±SD)	12.8(10.2)	10.5(8.2)	6.4(2.1)	0.503
Death, n(%)	1(20%)	2(15.4%)	0	

The comparison of patients with severe inhibition < 10% compared to moderate inhibition 10-30% and no inhibition > 30% showed that patients with more inhibition had greater rate of severe poisoning (p=0.002), mechanical ventilation (p=0.01), intermediate syndrome, atropine dose and duration of intermediate syndrome, mechanical ventilation and hospitalisation although the latter outcomes did not show statistical significance between the three groups. Overall, Table 5 shows that the baseline AChE level correlates with the clinical profile and that patients with severe inhibition of AChE have more severe clinical presentation with poorer outcomes than those with higher AChE levels.

TEMPORAL PROFILE OF AChE LEVEL AND CORRELATION WITH SEVERITY

The temporal profile of AChE among the severity groups (according to Namba scale) was assessed. The mean AChE value at presentation for the mild poisoning group was 75.92% of normal and was significantly higher than mean AChE of the moderate and severe poisoning group of 34% (p=0.020). The temporal profile of AChE in the mild poisoning group showed mild inhibition throughout the first 5 days compared to moderate and severe poisoning groups which had sustained inhibition throughout the duration of the study period. The temporal profiles of the moderate and severe poisoning groups were comparable with no significant difference. (Figure 9)

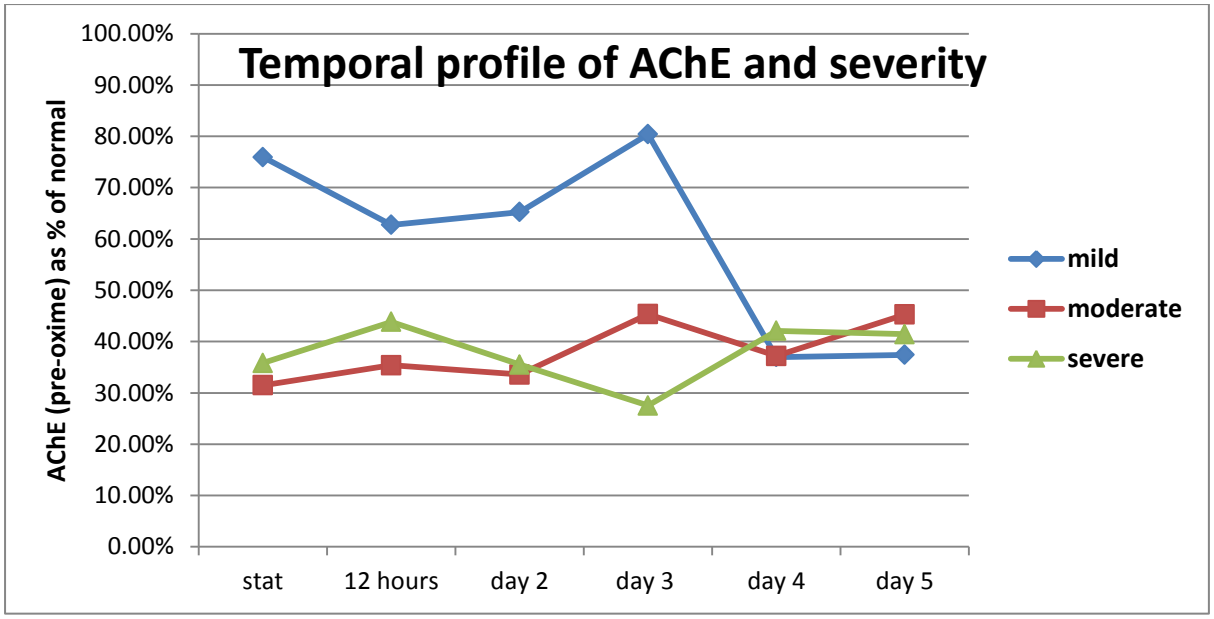


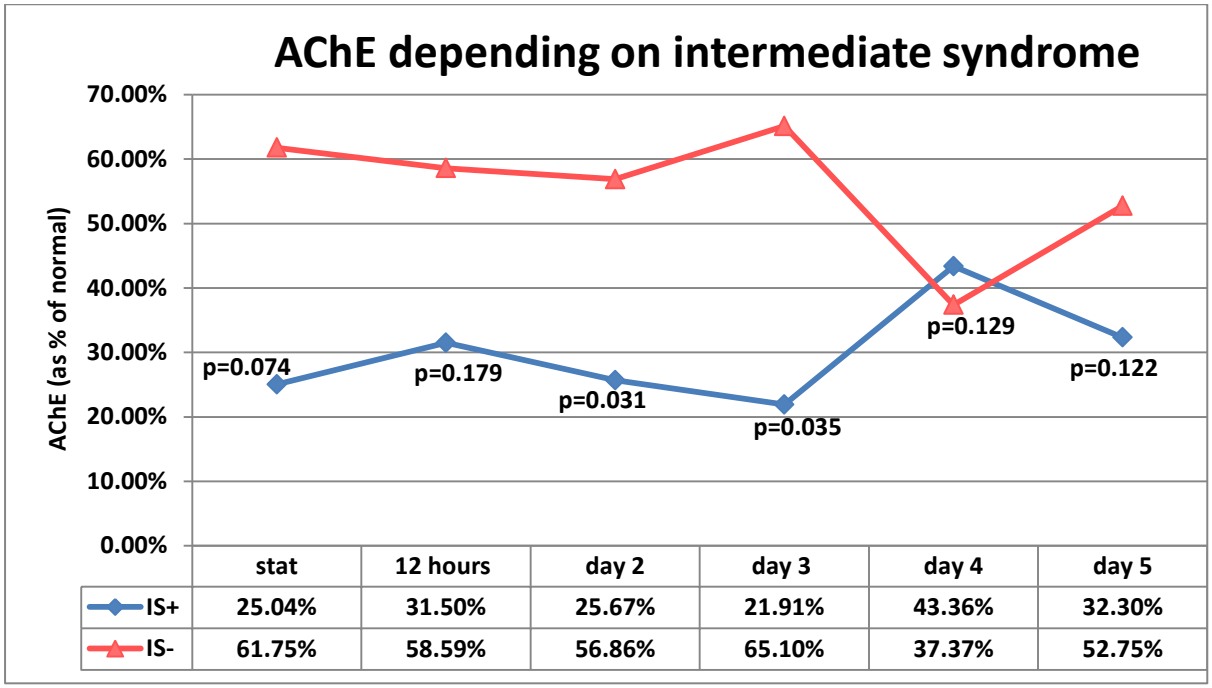
Figure 9: Temporal profile of AChE levels according to severity. The mild group had 11 patients, the moderate group 7 patients and the severe group had 12 patients.

AChE TEMPORAL PROFILE AND INTERMEDIATE SYNDROME AND MECHANICAL VENTILATION

There were 10 patients who developed intermediate syndrome and the temporal profile of their AChE levels was compared to that of the 20 patients who did not develop intermediate syndrome. The figure 10 below shows that the AChE levels of the intermediate syndrome group remain persistently inhibited compared to those who did not develop intermediate syndrome. The difference in baseline AChE in patients with intermediate syndrome compared to those who did not develop intermediate syndrome tended towards significance (p=0.074) and was significant on day 2 and 3.

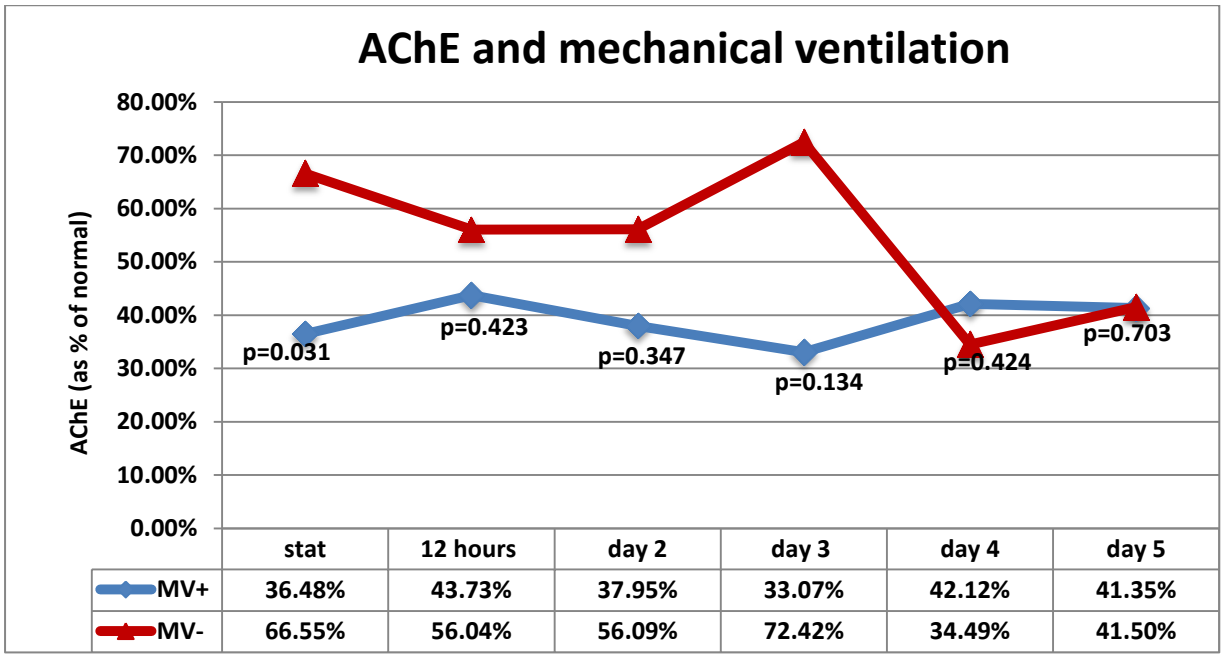
Similar pattern was seen in the 17 patients who required mechanical ventilation compared to the 13 patients who did not require ventilation (Figure 11). The

difference in baseline AChE in patients who required mechanical ventilation compared to those who did not was significant (p=0.031)



	stat	12 hours	day 2	day 3	day 4	day 5
IS+	25.04%	31.50%	25.67%	21.91%	43.36%	32.30%
IS-	61.75%	58.59%	56.86%	65.10%	37.37%	52.75%

Figure 10: Temporal profile of AChE levels among patients who developed intermediate syndrome (n = 10) compared to those who did not develop intermediate syndrome (n = 20)



	stat	12 hours	day 2	day 3	day 4	day 5
MV+	36.48%	43.73%	37.95%	33.07%	42.12%	41.35%
MV-	66.55%	56.04%	56.09%	72.42%	34.49%	41.50%

Figure 11: Temporal profile of AChE levels among those who required mechanical ventilation (n = 17) compared to those who did not require mechanical ventilation (n = 13)

AChE LEVELS AND COMPOUND TYPE

Compounds consumed were categorised according to their chemical structure and their baseline AChE values were analysed temporally. There were 4 categories as follows: Dimethyl compounds (12 patients), Diethyl compounds (10 patients), S-Alkyl compounds (4 patients) and Unknown compound (4 patients). There was no significant difference in the AChE values between the 3 groups (p=0.757). However the apparent mean AChE was higher in the S-alkyl compounds. (Figure 12)

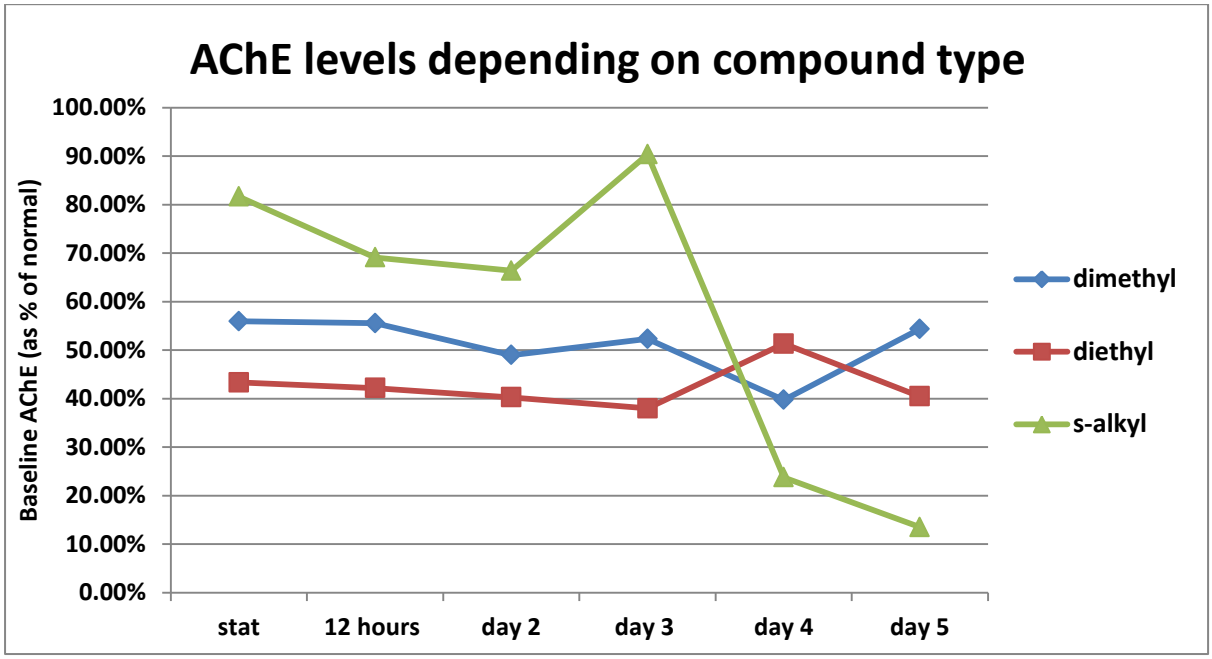


Figure 12: Temporal profile of AChE level among the dimethyl (n = 12), diethyl (n = 10) and S-alkyl (Profenofos) compounds (n = 4)

AChE AND TIME TO PRESENTATION (Figure 13)

It is expected that the AChE levels would be higher in those who presented earlier compared to those who present late. Based on this, patients were divided into 2 groups for analysis based on the lag time to presentation to CMC, Vellore:

1. Group A – Presented within 6 hours of consumption (19 patients)

2. Group B – Presented after 6 hours of consumption (11 patients)

Patients who presented early had significantly higher baseline AChE value at presentation at 66.5% of normal compared to those who presented late at 22.2% of normal as shown in figure 13 (p value = 0.018). Moreover, their mean AChE values over time also remained higher than the late presenters and this difference tended towards significance up to day 3 (day 2 p=0.061 and day 3 p=0.053).

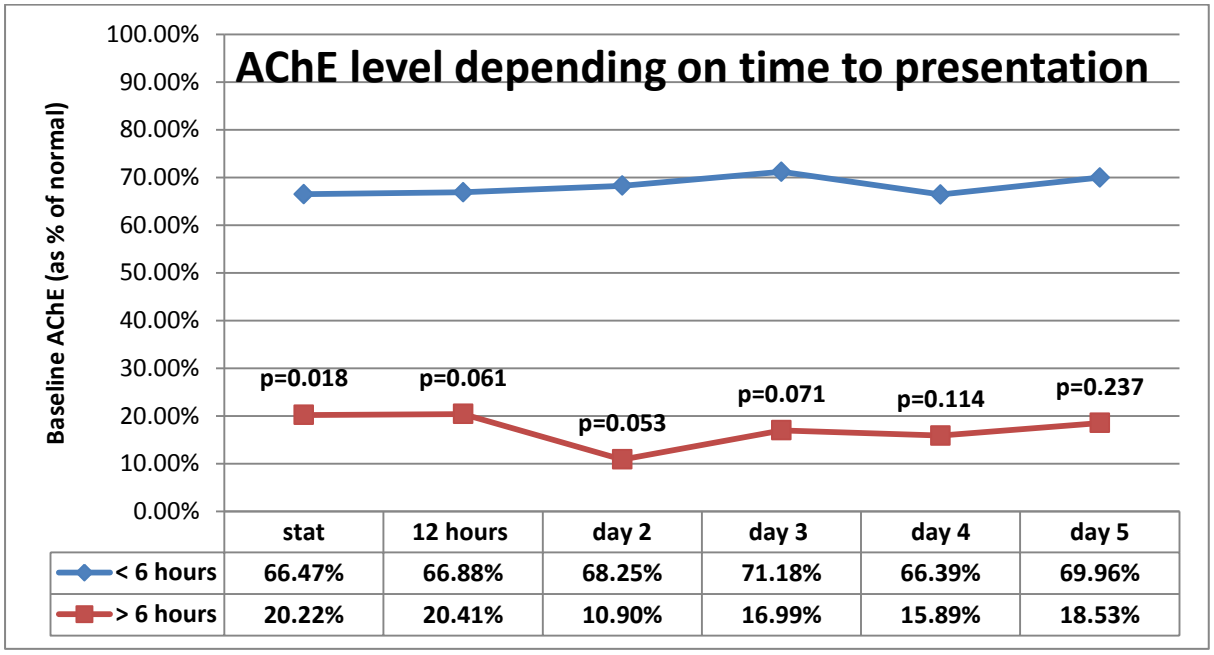


Figure 13: Temporal profile of AChE among the early (n = 19) and late presenters (n = 11)

AChE LEVEL AND PRIOR OXIME THERAPY (Figure 14)

Of the 30 patients, 20 had received treatment outside prior to arrival in CMC and 6 of these patients had documentation detailing the administration of prior pralidoxime (PAM) therapy elsewhere. None of the oxime doses were of adequate therapeutic dose based on WHO dosing schedule. The AChE in these two groups was also compared temporally. The mean AChE levels were lower in those who received prior oximes but this difference was not found to be significantly different (Figure 14).

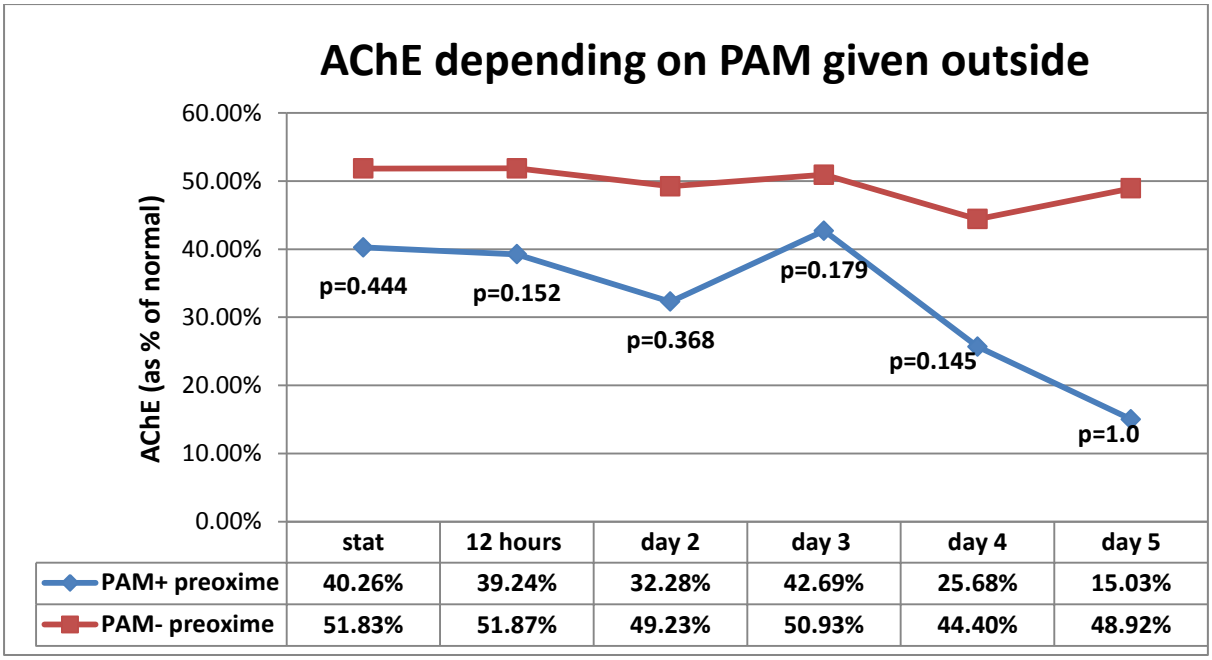


Figure 14: Temporal profile of AChE among those who received PAM elsewhere (n = 6) compared to those who did not receive PAM (n = 24)

REACTIVATION STATUS AND CLINICAL PROFILE OF OP POISONING

AChE (baseline) and AChE after addition of obidoxime to blood sample (post-reactivation) were measured daily in OP poisoning patients for 5 days. In order to analyse the post-reactivation AChE values, the 30 OP poisoning patients were first sub-divided based on their baseline AChE level at presentation. Those patients with no significant inhibition, namely those whose baseline levels remained more than 30% throughout the course of their admission, were excluded from the analysis. This group consisted of 8 patients. The remaining 22 patients with inhibited baseline AChE levels (<30%) were further sub-divided based on the post-reactivation AChE at presentation.

1. **Group 1 – Reactivation present (13 patients)**

- Included patients with AChE rising to above 30% at presentation after in vitro reactivation with oxime

2. **Group 2 – Reactivation absent (9 patients)**
    - Included patients with no significant rise in AChE (AChE persistently < 30%) even after in vitro reactivation with oxime
3. **Group 3 – No inhibition of AChE (8 patients)**
    - Baseline AChE > 30% throughout admission
    - This group was excluded from analysis regarding reactivation

Table 6: Clinical characteristics of groups according to the reactivation status

Clinical characteristics	Group 1 (n=13) – Reactivation present	Group 2 (n=9) – No reactivation	p value
Lag time to presentation to CMC – hours (±SD)	6.04(3.96)	10.94(8.06)	0.007
Prior oxime therapy, n(%)	3(23.2%)	2(22%)	0.518
Compounds			0.084
Dimethyl, n(%)	2(15.3%)	5(55.5%)	
Diethyl, n(%)	6(46.1%)	0	
Severity, n(%)			0.533
mild	6(46.1%)	0	
moderate	4(30.7%)	2(22.2%)	
severe	3(23.2%)	7(77.7%)	
Intermediate syndrome, n(%)	5(38.5%)	4(44.4%)	0.199
IS duration – days (±SD)	7.0(4.7)	9.25(6.2)	0.905
Mechanical Ventilation, n(%)	6(46.1%)	8(88.8%)	0.557
Ventilation duration – days	7.37(4.79)	8.7(3.8)	0.755
Atropine dose (mg)	139.2	480.5	0.209
Atropine duration – days (±SD)	3.15	3.33	0.896
Hospitalisation duration–days (±SD)	9.69	11.33	0.431
Death, n(%)	0	3(33.3%)	

The group with reactivation had presented significantly earlier (mean of 6 ± 4 hours) compared to the group without enzyme reactivation (mean of 11 ± 8 hours) (p=0.007). In the identified compounds, all the 6 diethyl compounds showed significant reactivation. In contrast, 5 out of 7 dimethyl compounds showed no reactivation. This difference tended towards statistical significance (p=0.084) Patients belonging to the group without reactivation required mechanical ventilation more frequently than the reactivation group of patients. Their mean duration of intermediate syndrome, ventilation and hospitalisation and mean atropine dose required were higher although these did not reach statistical significance. The need for mechanical ventilation and intermediate syndrome did not appear to be associated with the reactivability status. All 3 of the deaths in the study occurred in those patients with no reactivation with one death each occurring with monocrotophos and dimethoate (and one patient with unknown compound).

Among the 13 patients who showed significant in-vitro post-reactivation level more than 30%, the mean reactivation AChE level was found to be 86.8% of normal. The mean magnitude of rise in AChE was 61.8% from baseline after addition of oxime to the sample. In these patients with significant reactivation, obidoxime was able to reactivate the enzyme to more than 30% for a mean duration of 2.35 days (S.D. ± 1.7 days).

The mean post-reactivation AChE levels (expressed as % of normal) were plotted temporally for the ‘Reactivation’ and ‘No reactivation’ groups as described earlier (Figure 15). Among the group that showed significant reactivation, obidoxime

was able to reactivate AChE to a level of more than 30% up to day 3. There was significant difference in the mean post-reactivation AChE levels of groups 1 and 2 until day 3. Those who did not have reactivation on day 1 continued to have non-reactivable enzyme on subsequent days also. The number of patients with mean post-reactivation AChE levels > 30% of normal progressively reduced with 13 cases on day 1 with reactivability (>30%) compared to 9 cases on day 4 probably due to aging.

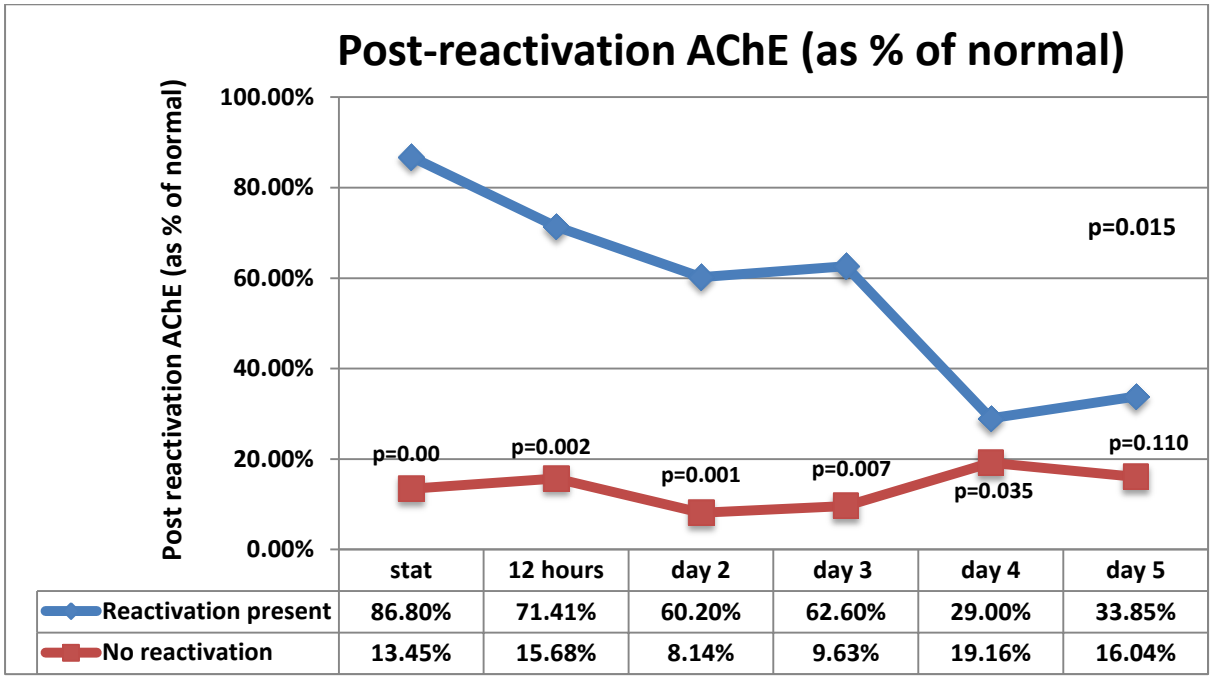


Figure 15: Temporal profile of post-reactivation AChE in the 'Reactivation present' group (n = 13) and the 'No reactivation' group (n = 9)

REACTIVATION STATUS AND TIME TO PRESENTATION

The patients were divided into two groups based on the lag time (cut-off of 6 hours) similar to the baseline AChE studies. In patients who presented early, obidoxime was able to reactivate the enzyme to more than 90% on the first 2 days and to more than 80% on the next 3 days. The baseline reactivation in early presenters (<6 hours) was 96.82% of normal compared to 52.88% in late presenters (>6 hours)

(p=0.037) (Figure 16). However, it must be noted here that the baseline values were also higher for early presenters compared to later presenters. (Figure 16)

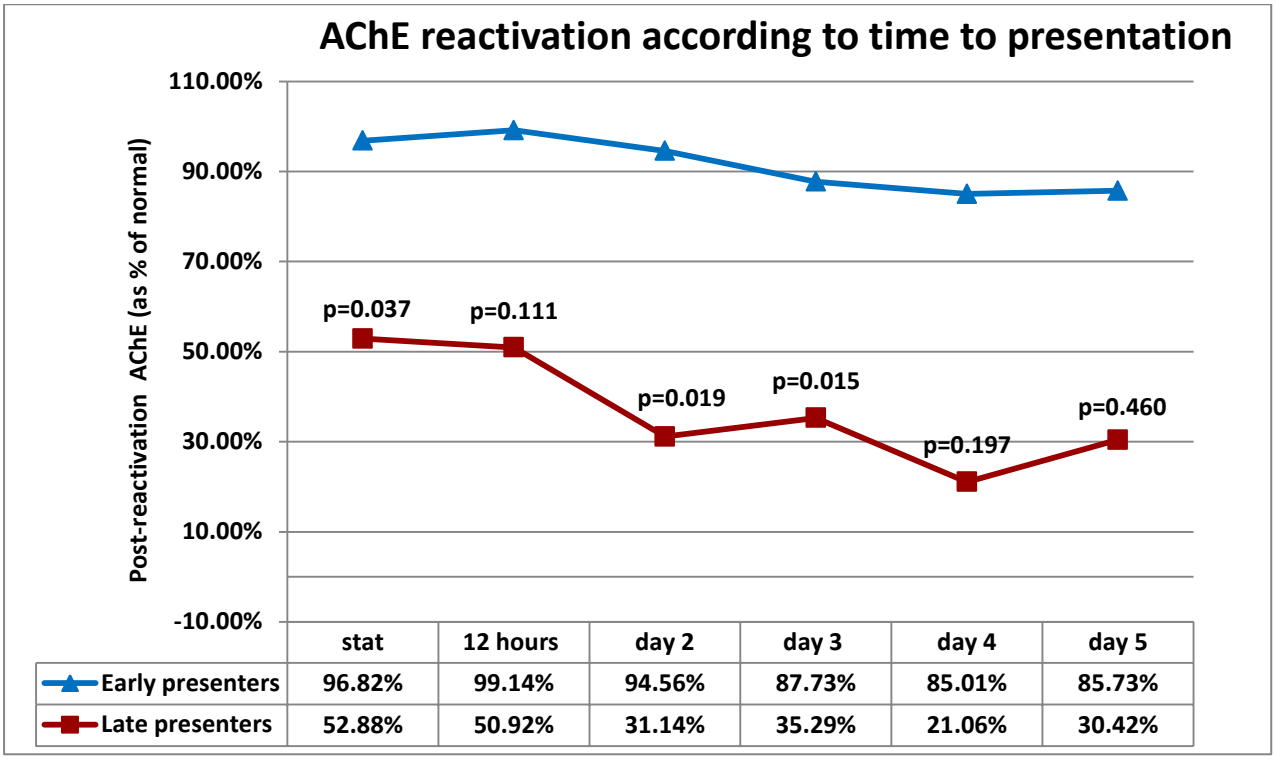


Figure 16: Temporal profile of post-reactivation AChE among the early presenters (n = 19) and the late presenters (n = 11)

REACTIVATION STATUS AND OP COMPOUND TYPE

OP compounds can be classified into dimethyl, diethyl and S-alkyl compounds depending on their chemical structure and these groups have different characteristics including ageing kinetics. The level of AChE after addition of oximes was analysed in the 2 major groups of dimethyl and diethyl compounds. The post-reactivation AChE values of the diethyl poisoning group were persistently higher than that of the dimethyl poisoning group but did not reach statistical significance. Day 1 and day 2 post-reactivation levels were 101% and 95% respectively in the diethyl compound group compared to 65% and 69% in the dimethyl compound poisoning group (p value

= 0.16) despite lower baseline AChE levels in the diethyl group compared to the dimethyl group (Figure 18).

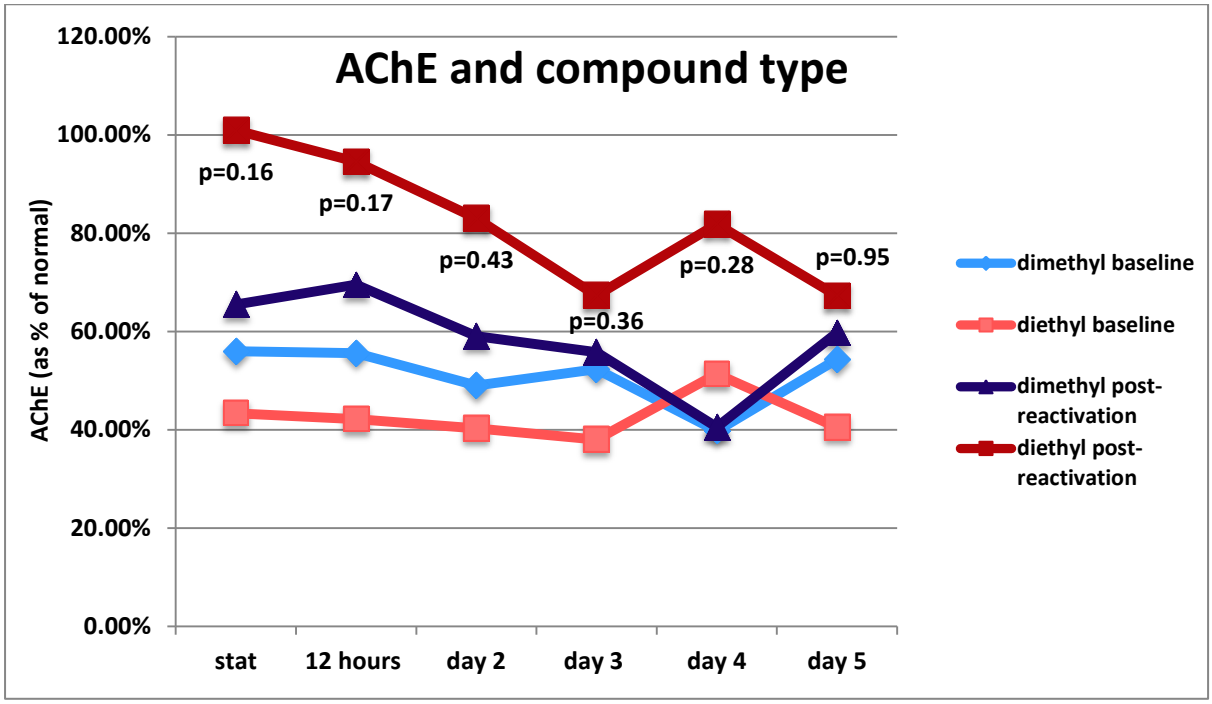


Figure 17: Temporal profile of Baseline and Post-reactivation AChE among the dimethyl (n = 12) and diethyl (n = 10) compounds

The magnitude of reactivation of AChE with oximes (expressed as % of normal) was also significantly higher among the diethyl compounds compared to the dimethyl compounds and this difference was maintained till 4 days after consumption. Obidoxime was able to reactivate at least 30% of enzyme activity till 3 days after consumption for diethyl compounds.

Figure 18 below depicts the magnitude of reactivation of AChE with obidoxime in 5 major OP compounds. Diethyl compounds, Triazophos and Chlorpyrifos, showed significant reactivation to >30% activity compared to the dimethyl and S-alkyl compounds.

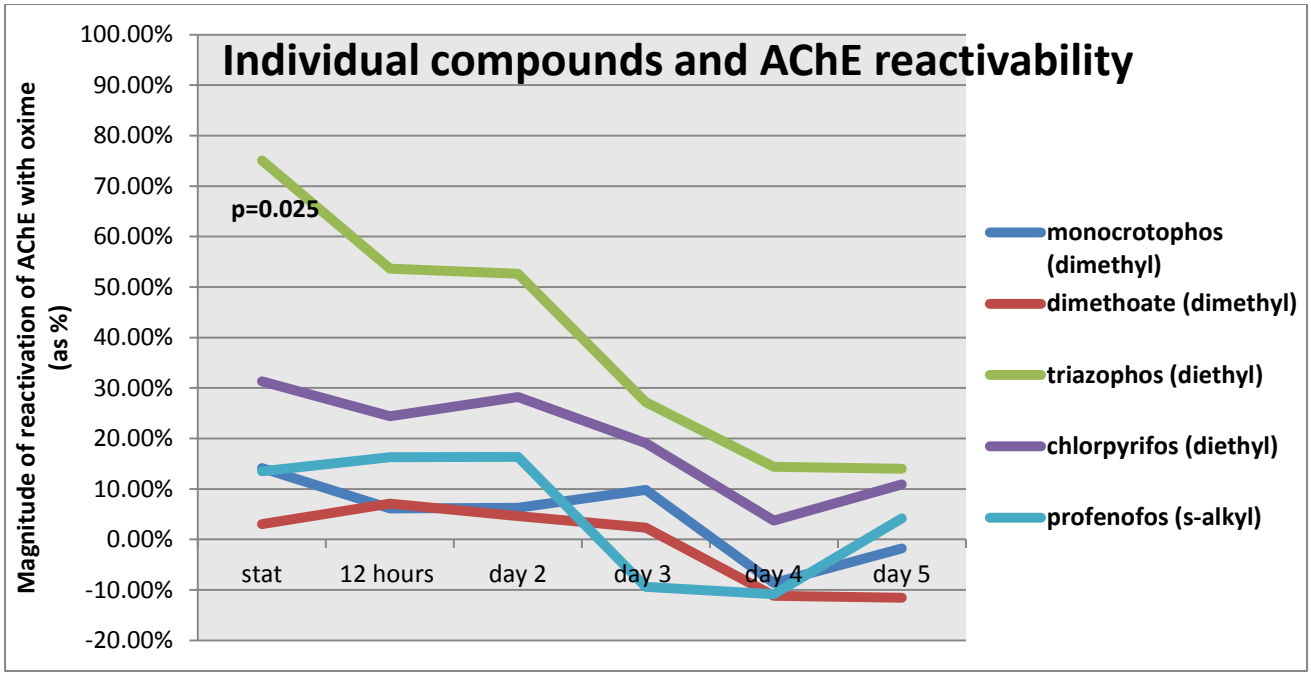


Figure 18: Magnitude of rise in AChE after addition of obidoxime for the major OP compounds

REACTIVATION STATUS AND INDIVIDUAL OP COMPOUNDS

Table 7: Oxime induced AChE reactivation for different OP compounds

Compound name	Reactivation groups – Post-reactivation AChE level		No inhibition of baseline AChE (n=8)
	>30% (n=13)	**<30% (n=9)**	
Unknown	2	2	0
Diethyl compounds			
Chlorpyrifos	2	0	0
Quinalphos	1	0	1
Triazophos	4	0	0
Phorate	1	0	1
Dimethyl compounds			
Monocrotophos	1	2	2
Dimethoate	1	3	0
Malathion	0	0	1
Methylparathion	0	0	1
Dichlorvos	0	0	1
S-Alkyl compounds			
Profenofos	1	2	1

In this study, there were a total of 10 different OP compounds distributed among the 30 patients. The distribution of these compounds among patient groups categorized based on reactivability is given in table 7. From this table, it was noted that the majority of the dimethyl (Monocrotophos and Dimethoate) and S-alkyl compounds (Propenofos) did not show reactivation compared to all the cases of diethyl compounds such as Triazophos and Chlorpyrifos which showed reactivation.

INHIBITORY ACTIVITY OF PATIENT PLASMA

The patient's plasma inhibitory activity levels were expressed as the percentage of normal control AChE remaining after addition of patient's plasma in vitro. This was done by calculating the AChE of a control sample before and after the addition of patient's plasma. This indicates the ability of the patient's plasma to inhibit control RBC-AChE and is a functional indicator of the amount of the OP compound in the patient's plasma. This test was done only on those patient samples in which the BChE was less than 5000U/L. Out of the 22 patients on whom this study was done, only 11 patients' plasma had the ability to inhibit control AChE to less than 90% of baseline at presentation. Among the remaining 11 patients, control AChE remained more than 90% of baseline. The clinical characteristics of the two groups are described in table 8 as follows:

Table 8: Clinical characteristics of compounds grouped according to inhibitory activity

Clinical characteristics	Group I – AChE inhibited to <90% of baseline n=11 (as % of n)	Group II – AChE remains >90% of baseline n=11 (as % of n)	p value
Lag time to presentation to CMC – hours (±SD)	9.45(7.89)	6.82(3.98)	0.65
Lag time < 6 hours, n(%)	5(45.45%)	6(54.55%)	0.68
Prior oxime therapy, n(%)	3(27.27%)	2(18.18%)	0.95
Compounds, n(%) dimethyl diethyl	 5(45.45%) 5(45.45%)	 4(36.36%) 3(27.27%)	0.64
Severity, n(%) mild moderate & severe	 1(9.1%) 10(90.91%)	 6(54.55%) 5(45.45%)	0.025
Intermediate syndrome, n(%)	5(45.45%)	4(36.36%)	0.39
IS duration – days (±SD)	8.80(6.26)	7.0(4.1)	0.41
Mechanical Ventilation, n(%)	13(72.2%)	4(33.3%)	0.67
Ventilation duration – days (±SD)	7.25(3.84)	9.20(5.1)	0.43
Atropine dose (mg)	390.1	165.3	0.056
Atropine duration – days (±SD)	3.82(1.17)	2.73(1.19)	0.076
Hospitalisation duration – days (±SD)	11.82(8.33)	9.64(7.06)	0.365
Death, n(%)	1(9.1%)	1(9.1%)	

Patients who had significant inhibitory activity at baseline (AChE after addition of patient plasma < 90%) had greater severity of poisoning (p=0.025) and higher

atropine dose (p=0.056). They were also associated with longer time to presentation and poorer outcomes in terms of need for mechanical ventilation, duration of atropine and duration of hospitalisation than the group without significant inhibitory activity though these difference did not reach statistical significance. The type of compound did not appear to have any relation to the inhibitory activity. Given the low level of inhibition of the control RBC AChE level, the significance of this assay is not clear.

INHIBITORY ACTIVITY AND CORRELATION WITH SEVERITY

The inhibitory activity of patients' plasma was studied across the severity groups (Figure 19) and as from previous Table 8, samples of patients with moderate and severe poisoning showed a significantly greater mean inhibitory activity of 83.6% compared to 115% in mild poisoning (p=0.016) . The inhibitory activity of <90% persisted in moderate and severe poisoning till day 4 although the difference was not statistically different.

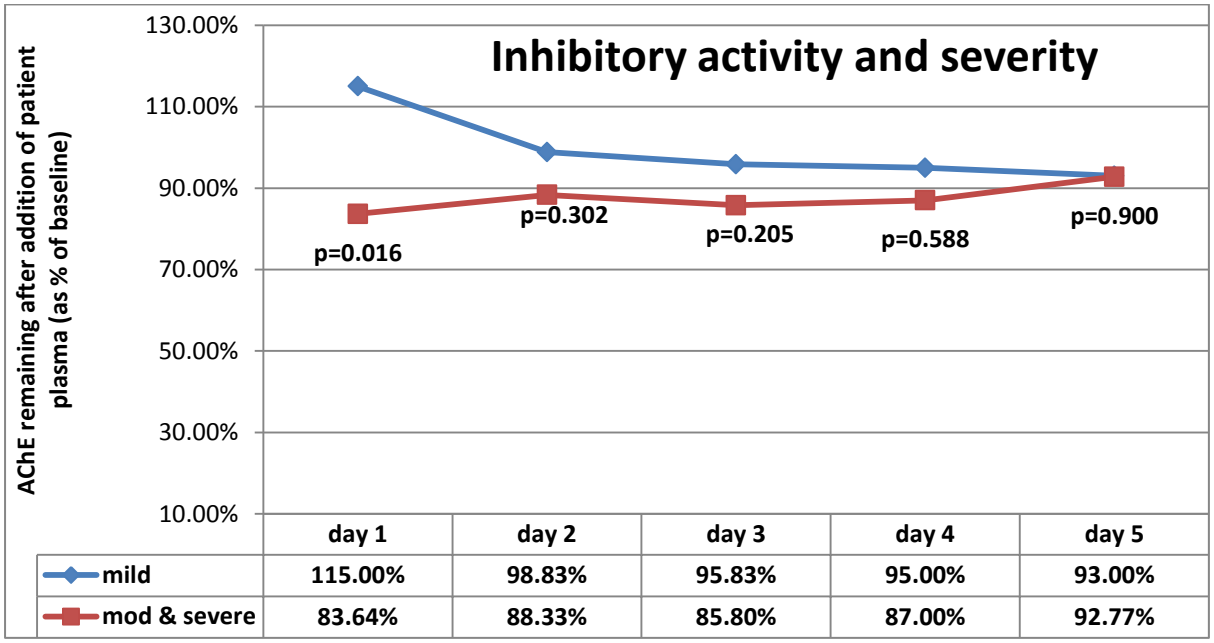


Figure 19: Temporal profile of inhibitory activity according to severity. The mild group has 7 patients and the moderate and severe group has 15 patients

INHIBITORY ACTIVITY AND CORRELATION WITH INTERMEDIATE SYDROME AND MECHANICAL VENTILATION

Among the 22 patients, 9 developed intermediate syndrome and the ability of their plasma to inhibit AChE was persistently greater compared to the group which did not develop intermediate syndrome but reached statistical significance only on day 2. Plasma of intermediate syndrome group of patients inhibited normal AChE to 83% and 79% on days 1 and 2 respectively compared to 101% (p=0.18) and 99% (p=0.02) for the group which did not have intermediate syndrome. (Figure 20)

13 of the 22 patients in this study required mechanical ventilation and their plasma inhibitory activity was persistently higher than the non-ventilated group but the difference was significant only on day 1 of poisoning. Control AChE was inhibited to a mean level of 82.7% of baseline by the plasma of the ventilated patients compared to 109.3% in the other group (p value = 0.028). (Figure 21)

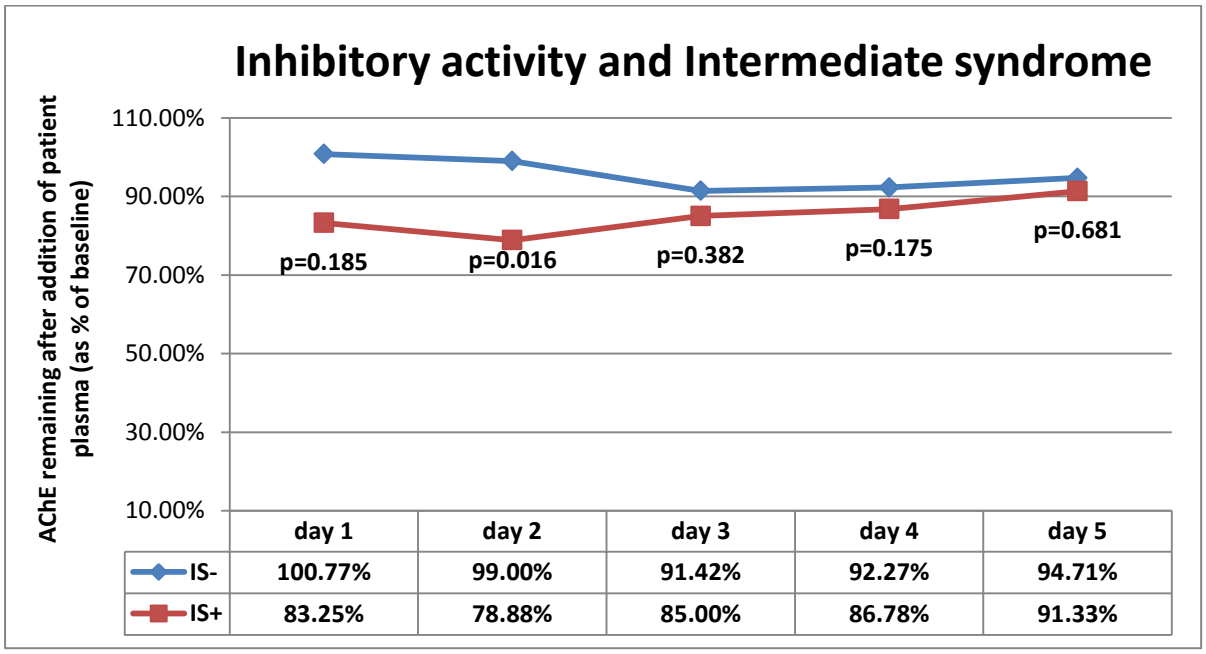


Figure 20: Temporal profile of plasma inhibitory activity of patients who developed intermediate syndrome (n = 9) compared to those who did not develop intermediate syndrome (n = 13)

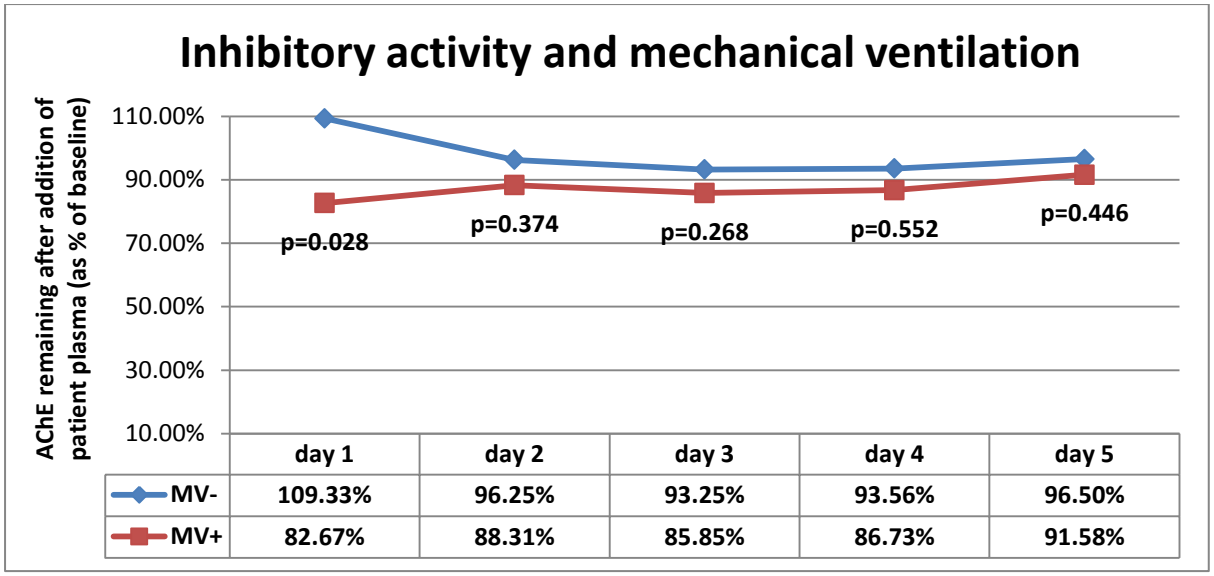


Figure 21: Temporal profile of plasma inhibitory activity of patients who required mechanical ventilation (n = 13) compared to those who did not require ventilation (n = 9)

INHIBITORY ACTIVITY AND TIME TO PRESENTATION

Out of 22 patients, 11 presented within 6 hours. Among these patients, their plasma, on an average had the capacity to inhibit control AChE to 84% compared to 103% for the late presentation group (p value = 0.314). However, there was no significant difference in the inhibitory activity of the two groups.

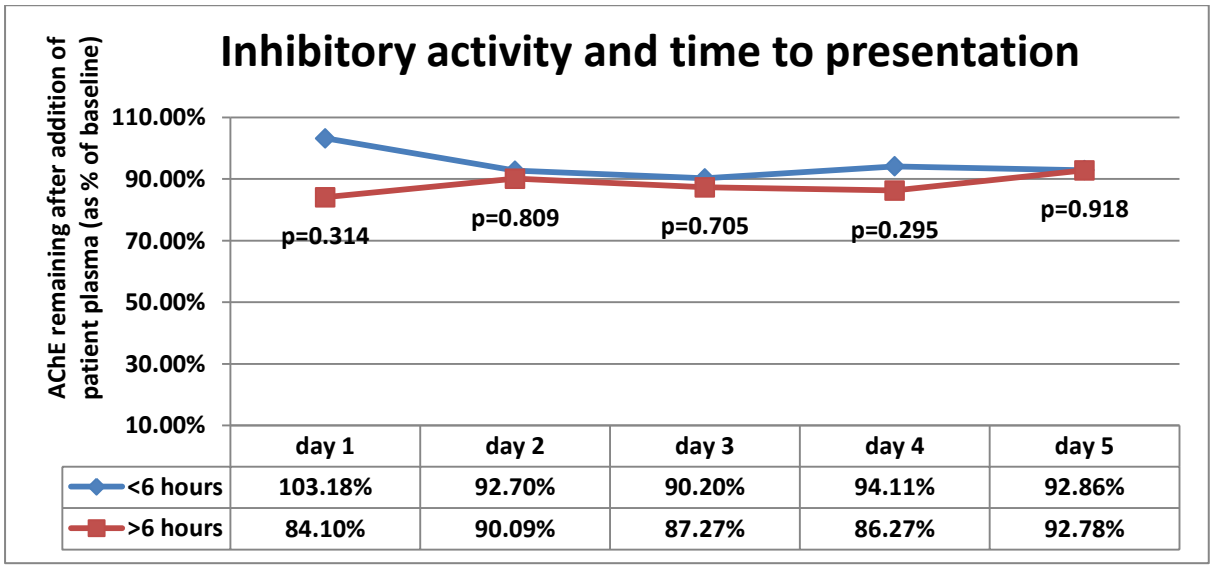


Figure 22: Temporal profile of plasma inhibitory activity among the early presenters (n = 11) and late presenters (n = 11)

RESULTS – (B) STUDY OF POISONING PROFILE USING THE POISON DATABASE

The results from the analysis of the poison database 2009-2014 was as follows:

DEMOGRAPHIC CHARACTERISTICS

A total of 2323 patients who presented with poisoning between July 2009 to June 2014 were evaluated. The mean age (±SD) was 30.47(12.23) with a range of 14 - 90 years. Almost half the population belonged to the young adult age group of 20-29 years. 81% of the patients were less than 40 years of age. (Figure 23)

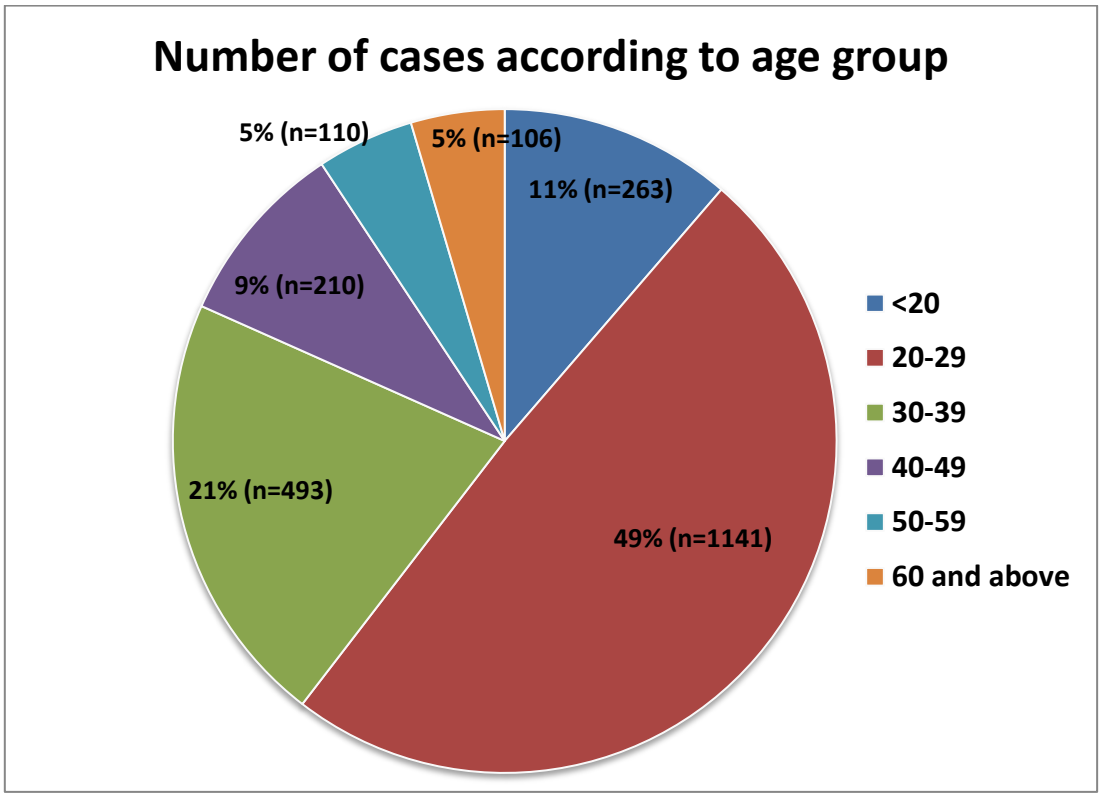


Figure 23: Number of poisonings according to age-groups

Among the patients with pesticide poisoning, this trend was maintained and about 75% of the population was below 36 years of age as depicted below in figure 27. (Figure 24)

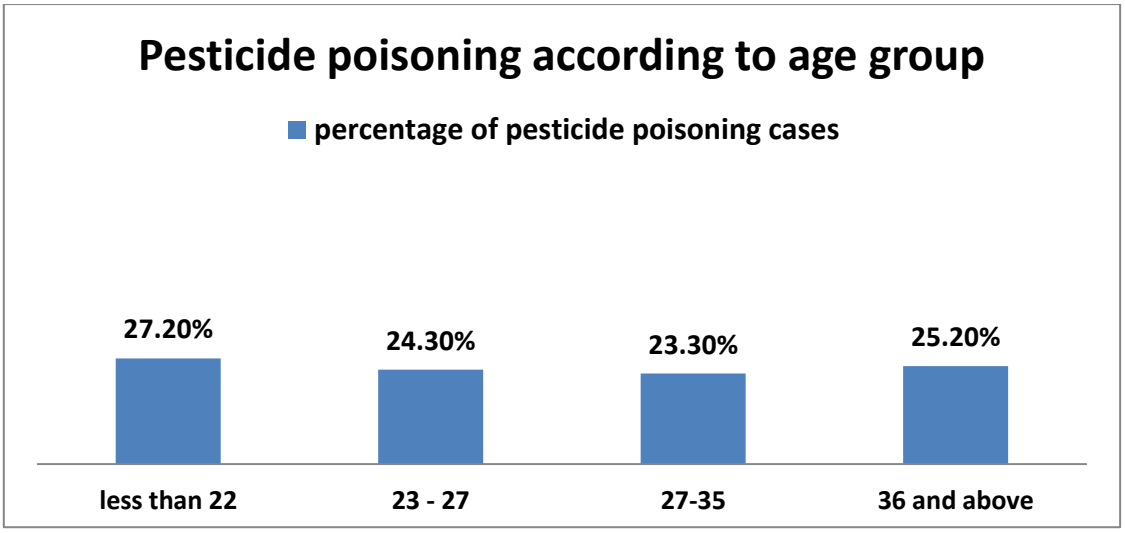


Figure 24: Pesticide poisoning according to age-group

There was a slight male predominance (52%) among all poisoning cases which was more prominent in pesticide poisoning (64.5%). However, there was a female predominance (66%) in the 635 cases of drug overdose. (Figure 25)

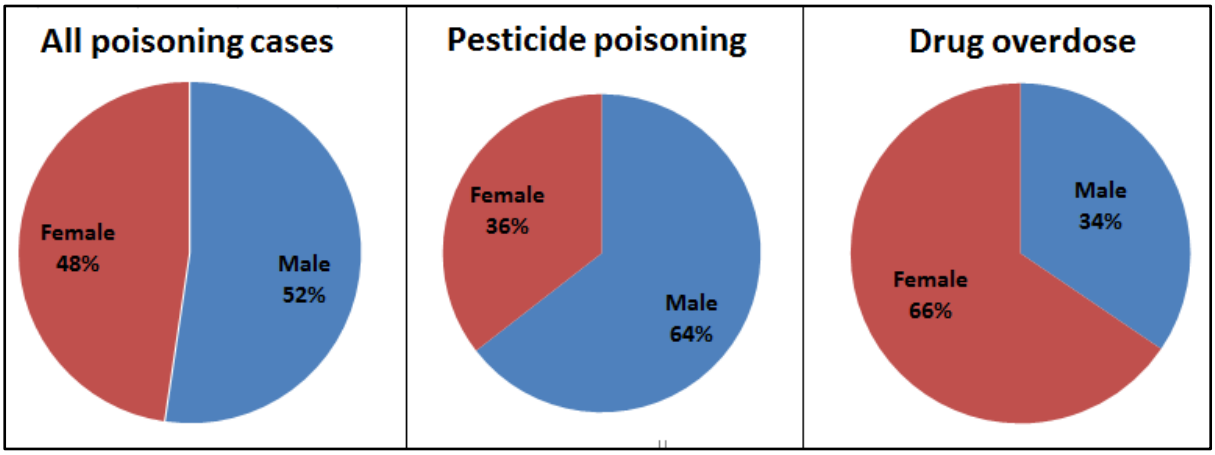


Figure 25: Sex distribution for all poisonings, pesticide poisoning and drug overdose

Most of the patients who presented to our centre were from Tamil Nadu (82.9) with most patients belonging to Vellore(62.9% of all poisoning cases) and Thiruvannamalai(16.9%) districts. 16.2% of all the poisoning patients were from the neighbouring state of Andhra Pradesh predominantly belonging to the districts of Chittoor and Kadapa.

Housewives accounted for 27% of poisonings of all causes with labourers being the largest occupation group accounting for one-quarter of the cases of poisoning. (Figure 26) In pesticide poisoning, farmers (17%) and labourers (32%) accounted for nearly half the cases while in plant poisoning, labourers and housewives were the predominant groups. Housewives (39%) and students (20%) constituted most of the cases of drug overdoses. The figure 27 highlights that pesticide poisoning followed by drug overdose is the most common method of poisoning among labourers, farmers and students and among housewives' the common method is drug overdose followed by pesticide poisoning.

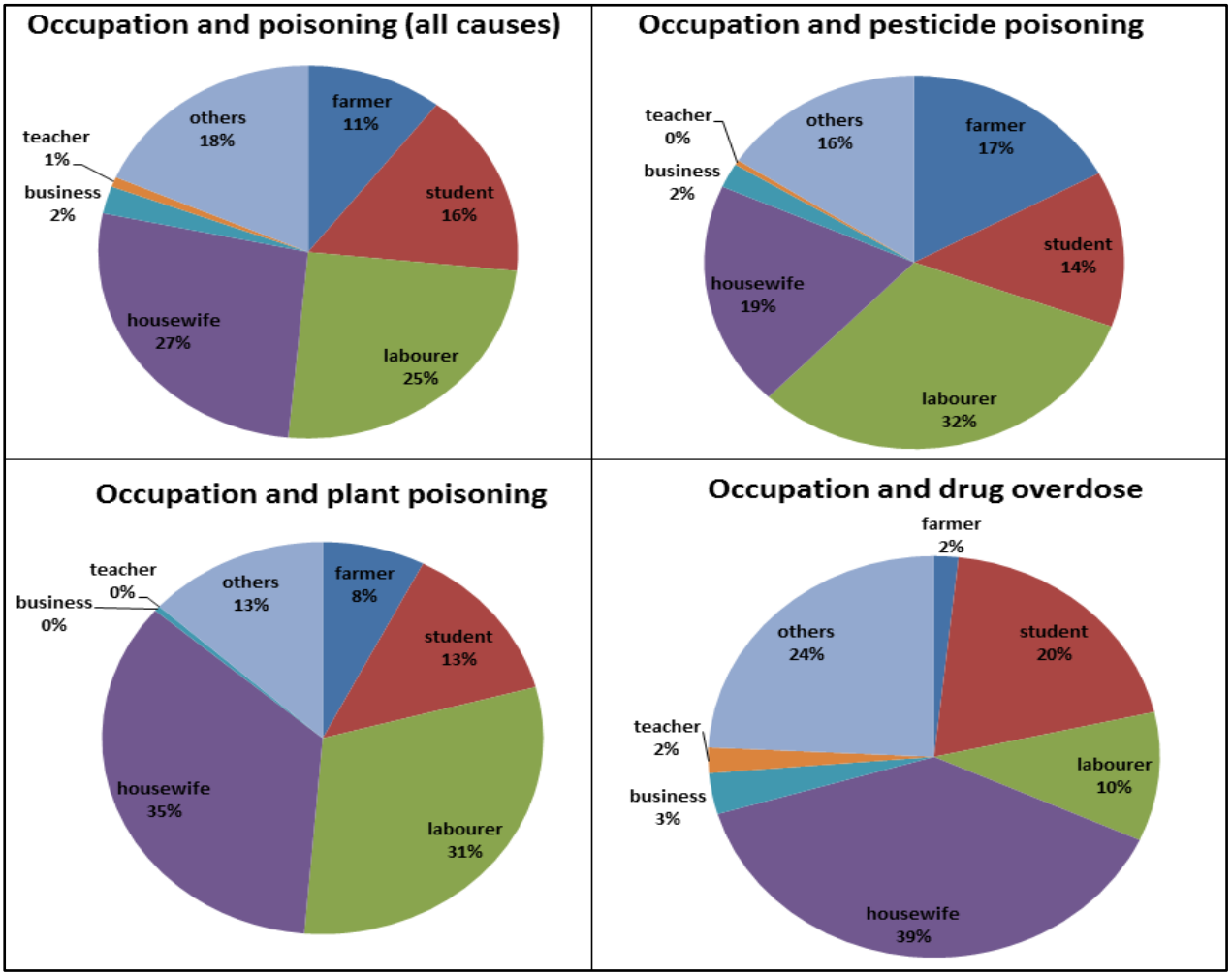


Figure 26: Compound used and occupation

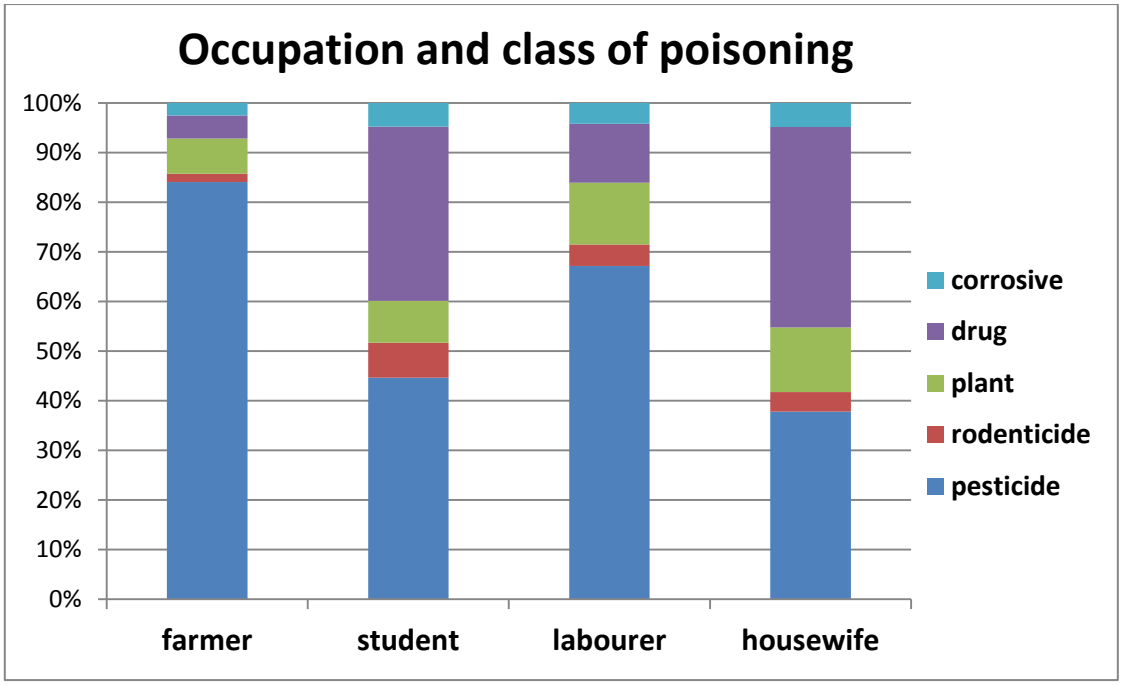


Figure 27: Occupation and class of poisoning

CLASS OF POISONS AND TIME TREND OF POISONING

Of the 2323 cases of poisoning, 28 cases were due to accidental poison ingestion with all the remaining cases presenting with deliberate self-harm. There had been no cases of homicide admitted in our centre during this period with poisoning. 88% consumed only a single compound with the rest consuming 2 or more compounds which were most often combination of different classes of drugs or mixed classes of poisons. 1178 among the 2323 cases were attributed to pesticide poisoning accounting for 50.7% of all poisonings. Drugs (27.3%) followed by plants (9.7%) were the next most common agents used for poisoning. Rodenticides were used in 4.4% of all poisonings and corrosives including hair dyes in 4%. (Figure 28)

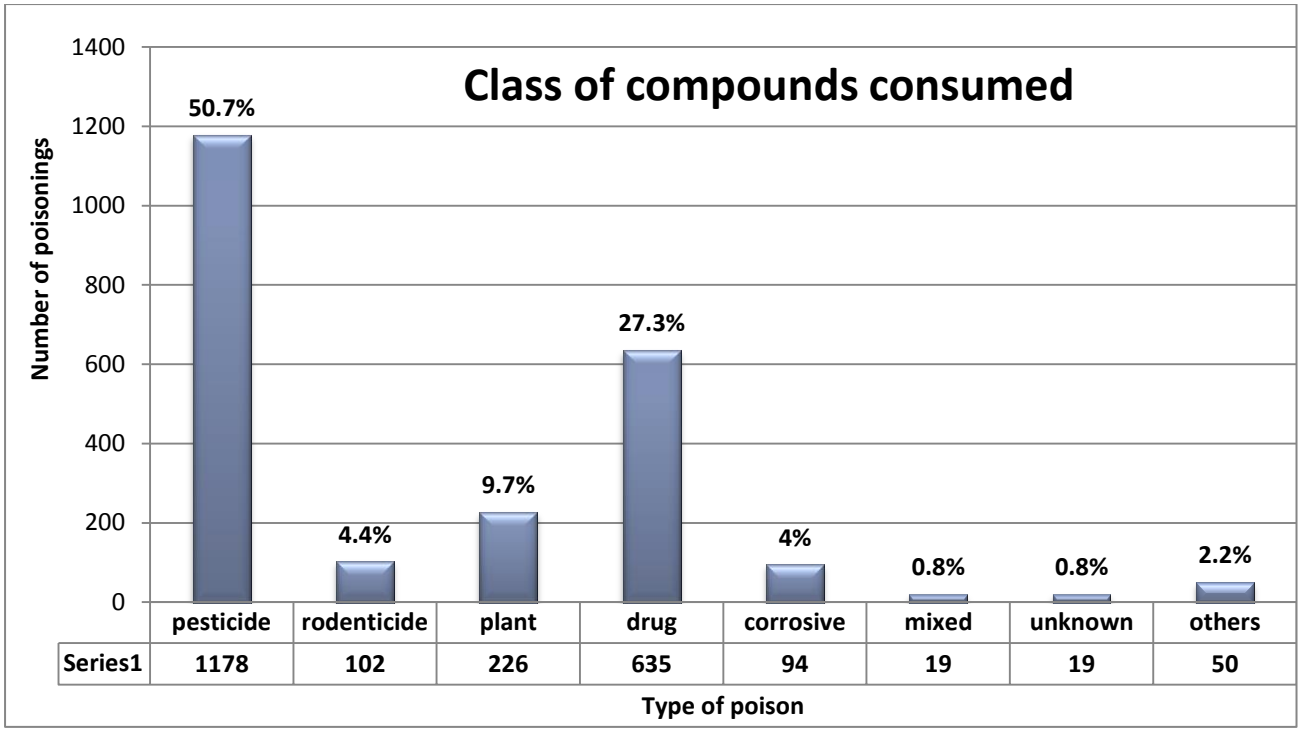


Figure 28: Numbers of poisoning of different classes

The overall percentages of the classes of compounds used for poisoning have remained stable over the years as evidenced in figure 29. Pesticides have accounted for about half the cases of poisoning every year between July 2009 to June 2014. Drugs have remained the second most common method of poisoning with percentages being slightly higher in 2012-13 and 2013-14 (28.6% and 27.1% respectively) compared to 2011-12(22.70%). The share of plant poisonings among all causes of poisoning has remained stable in the time period of the study ranging from 8.8% to 10.9%.

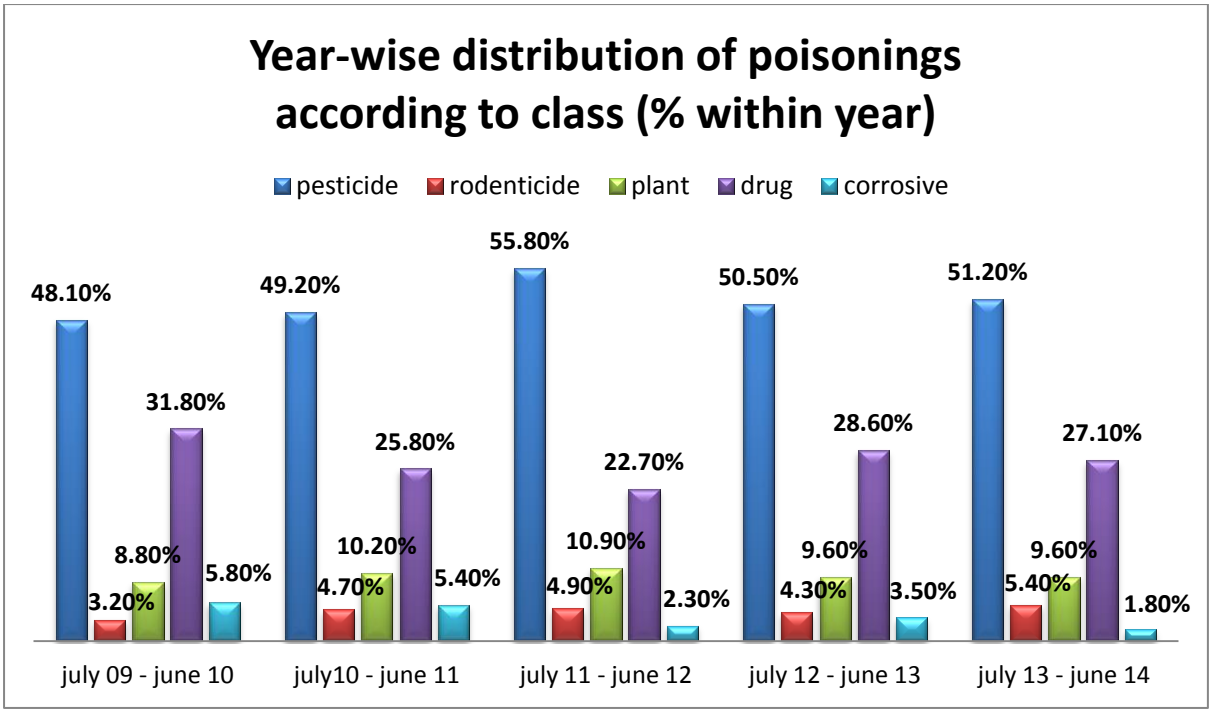
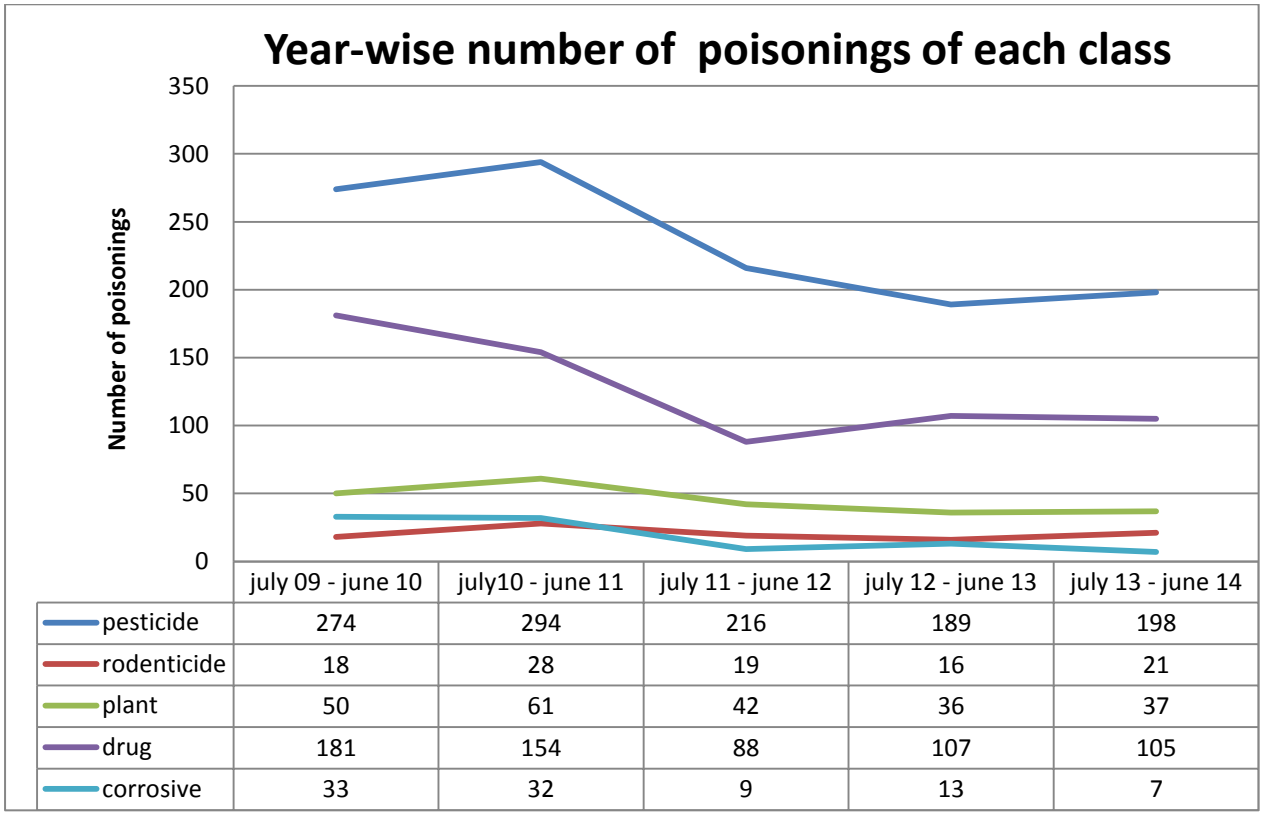


Figure 29: Proportion of different classes of compounds in each year

Analysis of the time trend of the number of poisonings in 2009-14 shows that there has been a marked decline in the overall numbers of all poisoning cases presenting to our institution which has been represented graphically in Figure 30. While there were 570 and 598 poisoning cases in 2009-10 and 2010-11 respectively, there were only 374 and 387 cases in 2012-13 and 2013-14 respectively. Each of the individual compound poisonings including pesticide, drug, plant and corrosive poisonings showed a marked decline over the 5 year time frame except for rodenticide poisoning, the numbers of which remained stationary. While the number of pesticide poisoning patients dropped from 274 in 2009-10 to 198 in 2013-14 (proportional fall of 27.7%), the numbers in the drug overdose group fell from 181 to 105 in this same period (proportional fall 42%). While the number of plant poisoning cases peaked at 61 in 2010-11, there has been a decline to 37 cases in the last recorded time period of 2013-14 (proportional fall of 39.3%). Further it is also clear that the decline of all

poisonings occurred in the period of 2011-12 compared to the period before this (2009-2011) (proportional fall 33.7%). In the period of 2012-2014, the number of poisonings and the proportion of different classes remained stationary. Therefore it is clear that there was a change in the admission pattern of poisonings during 2011-12 which affected the majority of poison compounds and this change has been sustained after that.



	july 09 - june 10	july10 - june 11	july 11 - june 12	july 12 - june 13	july 13 - june 14
pesticide	274	294	216	189	198
rodenticide	18	28	19	16	21
plant	50	61	42	36	37
drug	181	154	88	107	105
corrosive	33	32	9	13	7

Figure 30: Year-wise number of poisonings of each class

It appears from the above graph that there was a sharp decline in all poisons between 2010-11 and 2011-12. Since that time the number of each kind of poisoning has remained stationary. The number of poisonings during these years and the proportional decline of poisoning in this time period (expressed as a percentage of the 2010-11 numbers) is summarised below in table 9.

Table 9: Proportional decline in the number of different classes of poisonings in 2011-12 compared to 2010-11

Compound class	Number of cases in 2010-11	Number of cases in 2011-12	Proportional decline (as % of 2010-11) between 2010-11 and 2011-12
Overall	598	387	35.28%
Pesticide	294	216	26.53%
Rodenticide	28	19	32.14%
Plant	61	42	31.15%
Drug	154	88	42.86%
Corrosive	32	9	71.88%

PESTICIDE POISONING

Of the 1178 cases of pesticide poisoning, organophosphorus compounds were used in 50% of cases of which organophosphates were combined with pyrethroids in 8% of cases. Pyrethroids were associated with 23% of all pesticide poisonings. Organochlorines and Carbamates were the causative agents in 5% and 2% of patients respectively. In a quarter of the patients, the pesticide compound was not known. The pesticides were also classified according to the WHO class of toxicity and class II compounds which are under the moderately hazardous agents were the most common agents used, accounting for 42.6% of pesticide poisonings. The extremely and highly hazardous compounds of class Ia and Ib constituted 26.5% of pesticide poisonings. Among the individual compounds, chlorpyrifos and monocrotophos were the most common compounds used with 104 and 99 cases respectively. The class Ia compounds of phorate, methylparathion and parathion accounted for 60, 62 and 15 cases respectively.

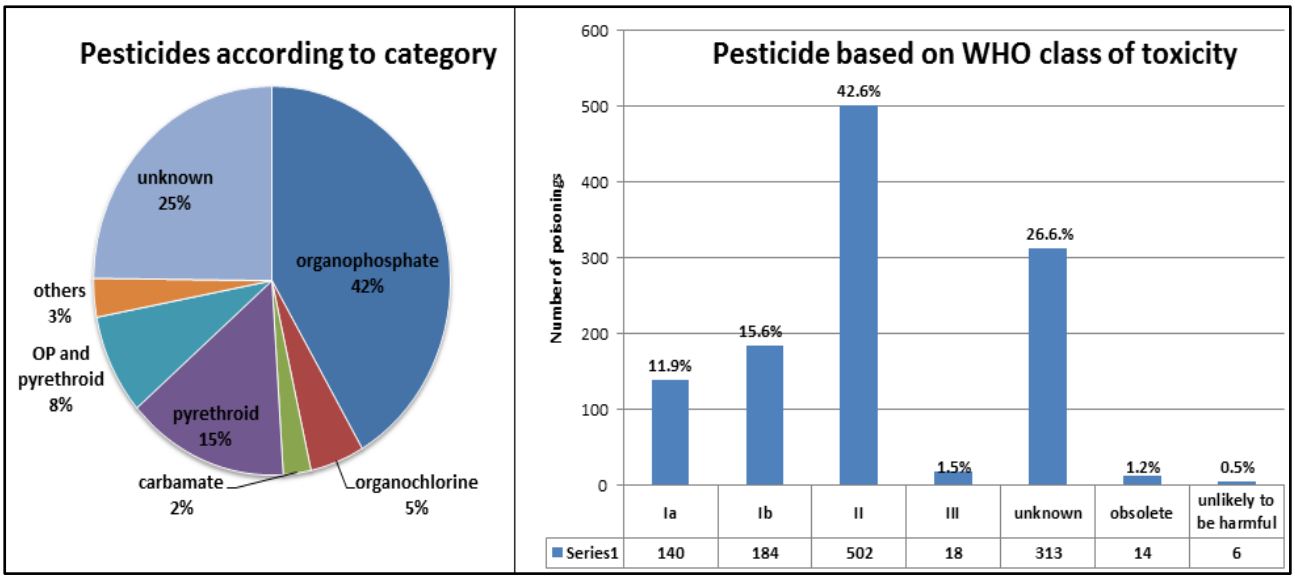


Figure 31: Pesticide poisoning proportion according to category and WHO class of toxicity

The temporal profile of pesticide poisoning was analysed according to the WHO class of toxicity of pesticide poisoning and the category of pesticides. There was a drop in overall numbers in all categories as well as the relative percentages of different categories. Class II compounds which constituted 38.3% of pesticide poisonings in 2009-10 accounted for 44.8% in 2013-14 constituting a 6% rise in proportions although there was a drop in absolute numbers from 104 to 87 cases. The class I compounds remained as the causative agents in about 30% of cases. When analysed by category, there was a rise in proportions between 2009-10 and 2013-14 of organophosphorus compounds (55.7% to 64.7%). The combined formulations of OP and pyrethroid which constituted 7.2% of pesticide poisonings in 2009-10 peaked to 16.4% in 2012-13 and again declined to 8.7% in 2013-14. There was a marked decline of proportion of organochlorine poisoning (11.86% in 2009-10 to 2.0% in 2013-14) and pure pyrethroid poisoning (21.1% in 2009-10 to 15.0% in 2012-13). The newer pesticides which could not be classified into any of these categories were less than 1% in all the years.

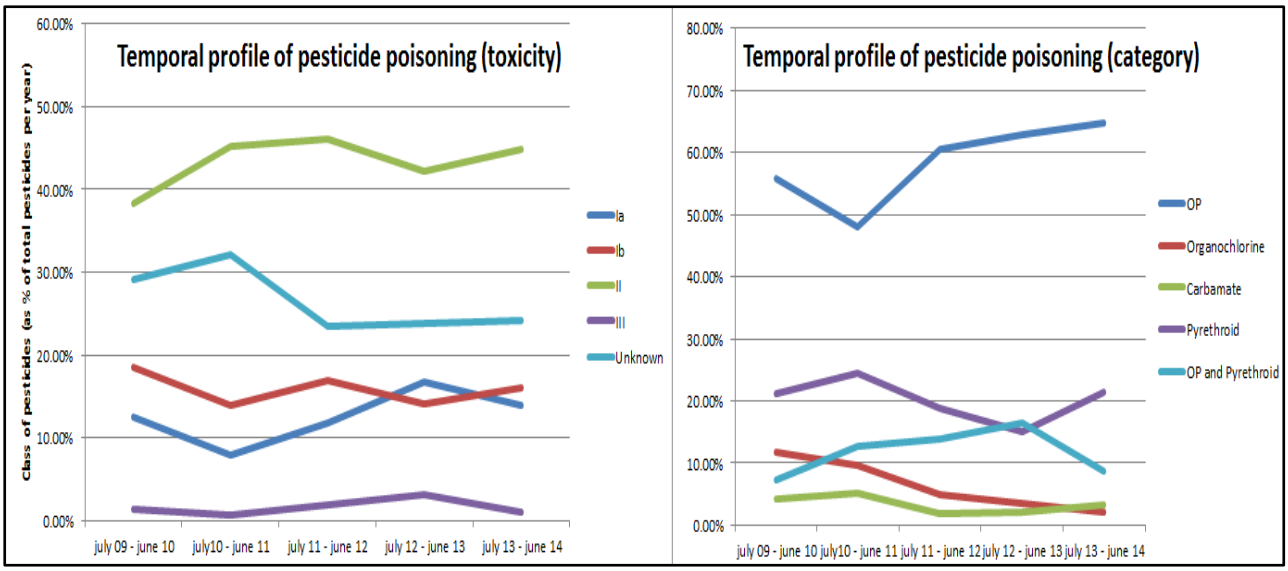


Figure 32: Temporal profile of pesticide poisoning according to category and WHO class of toxicity

ENDOSULFAN

Out of the 58 cases of organochlorine poisoning, there were 39 cases of the highly fatal endosulfan poison. There was a sharp decline in the number of endosulfan poisoning cases during the period of the study with 30 cases up to June 2011 and 9 cases thereafter. This decline was simultaneous to the national ban on Endosulfan in 2011. There were no cases of endosulfan poisoning after June 2013. The graph also shows that the mortality due to Endosulfan which was 29.4% was in 2009-2010 was completely eliminated which shows the efficacy of the ban and the impact of pesticide restriction on poisoning mortality. (Figure 33)

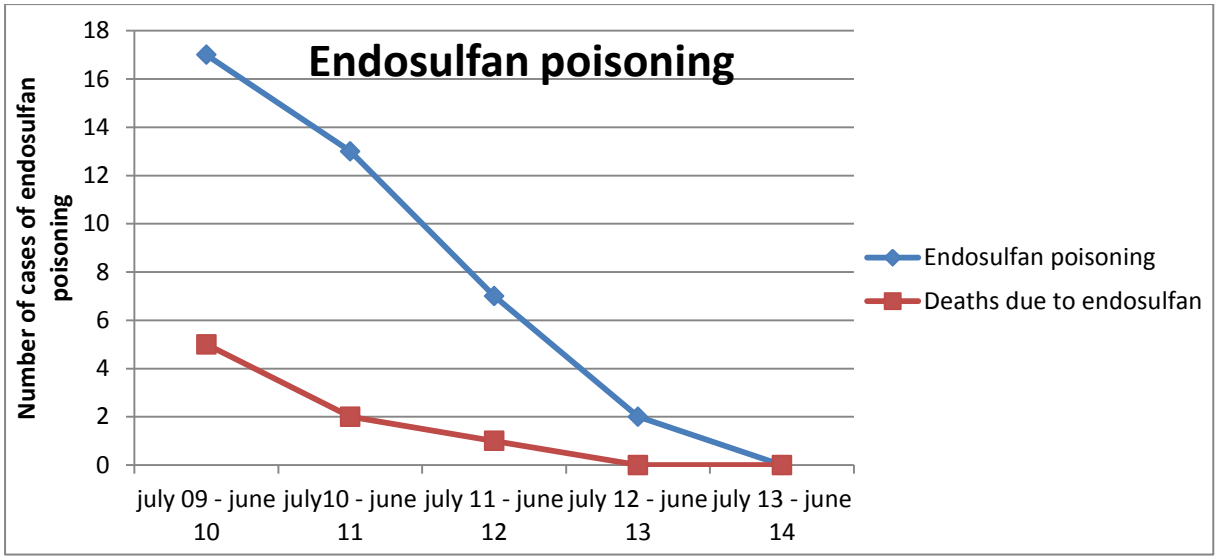


Figure 33: Temporal profile of endosulfan poisoning - number of cases and deaths due to endosulfan

RODENTICIDE POISONING

There were 102 cases of rodenticide poisoning in the 5 years of study with the compound not being known in 43% of cases. Zinc phosphide was the most common rodenticide followed by yellow phosphorous, coumarins and aluminium phosphide with percentages as depicted below (Figure 34)

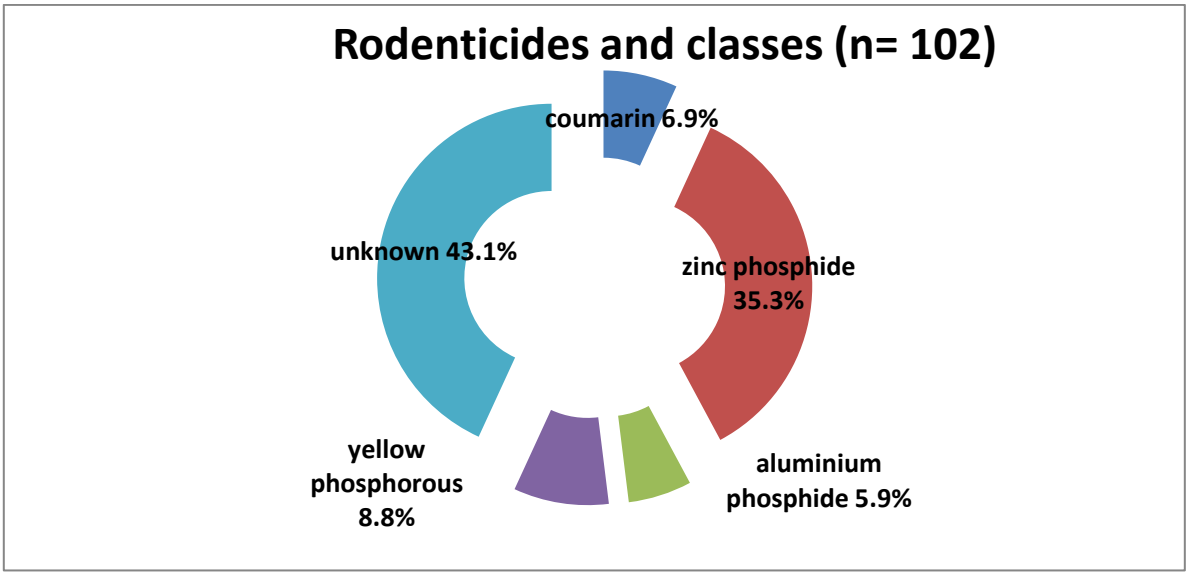


Figure 34: Proportions of different rodenticide compounds used

PLANT POISONING

Of the 226 cases of plant poisoning, there were 120 and 102 cases of yellow oleander and Oduvanthalai poisoning respectively. There was a significant fall in the number of plant poisonings with 111 cases in the first two years of study compared to 73 cases in the last two years of the study period. In comparison to 50 and 49 cases of oleander and oduvathalai poisoning in the 2009-11, there were 34 and 29 cases respectively in 2012-14. (Figure 35)

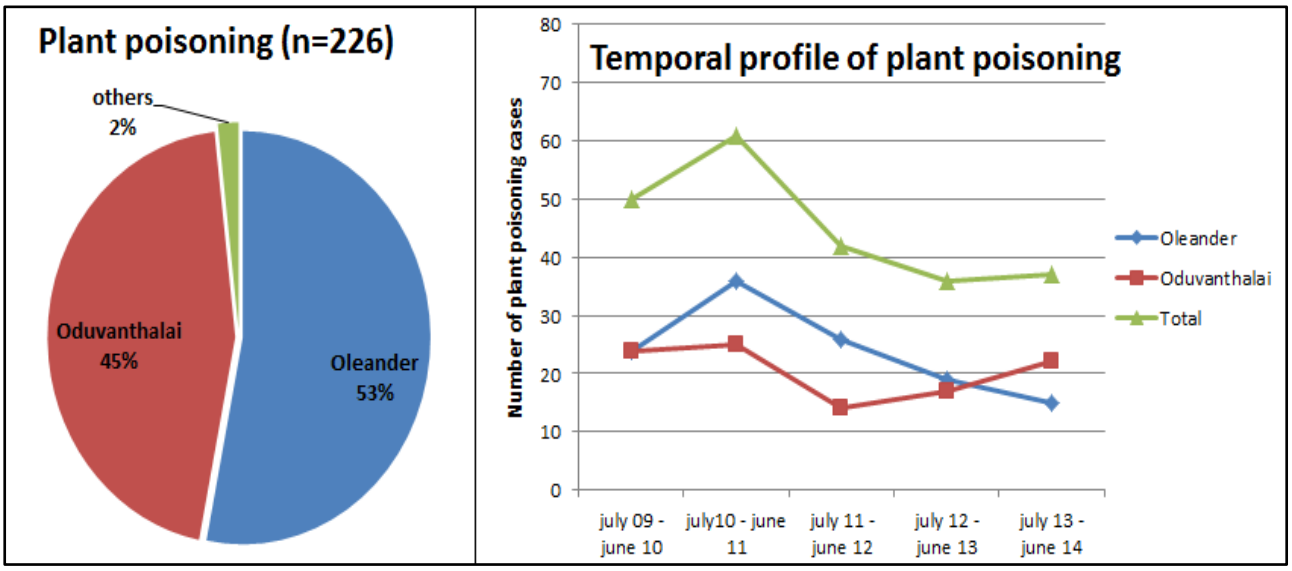


Figure 35: Proportions of different plant poisons used and the temporal profile of the number of plant (overall), oleander and oduvanthalai poisoning

DRUG OVERDOSE

There were 635 patients presenting with drug overdoses to our hospital during the study period of whom, 135 had consumed 2 or more different classes of drugs. In 69 patients, the offending drug was not known. The most common drugs used were benzodiazepines with 152 cases due to this class (constituting 24% of the drug overdose cases) with barbiturates being used in only 29 cases (4.5%). Other

antiepileptics were used in 61 cases (9.5%). Paracetamol and NSAIDs were used in 96 cases and antihistaminics in 63. Among the antipsychotics, atypical antipsychotics were more commonly used in 70 cases (11%) in comparison to 11 (1.7%) for typical antipsychotics. Other central nervous system agents such as antidepressants were the causative agents in 75 cases (11.8%). Poisoning due to all the major classes of drugs were on a downward trend similar to the trends of overall poisoning with no increase in any specific group. Other drugs had small numbers accounting for less than 20 cases.

CORROSIVES

There were 92 cases of corrosive poisoning during the study period with super vasmol hair dye being the most common agent used in 28% of cases. (Figure 36) There was a fall in overall numbers of corrosive poisoning from 65 cases in the first 2 years of the study period to 20 cases in the last 2 years. Phenol or Lysol toilet cleaners were used in 24% of cases and hydrochloric acid in 22%. There was a relative fall in the number of cases of super vasmol poisoning with only 7 cases in the last 2 years of the study period compared to 16 cases in the first 2 years.

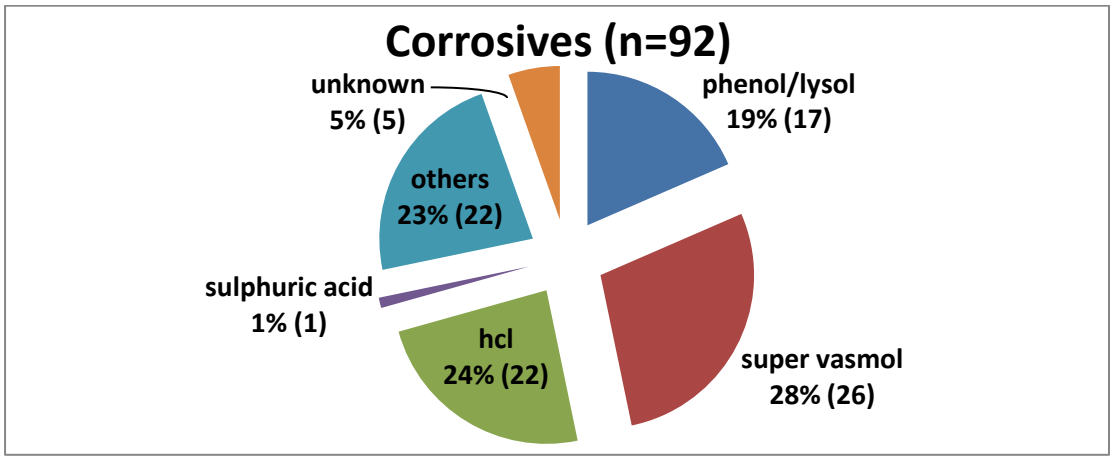
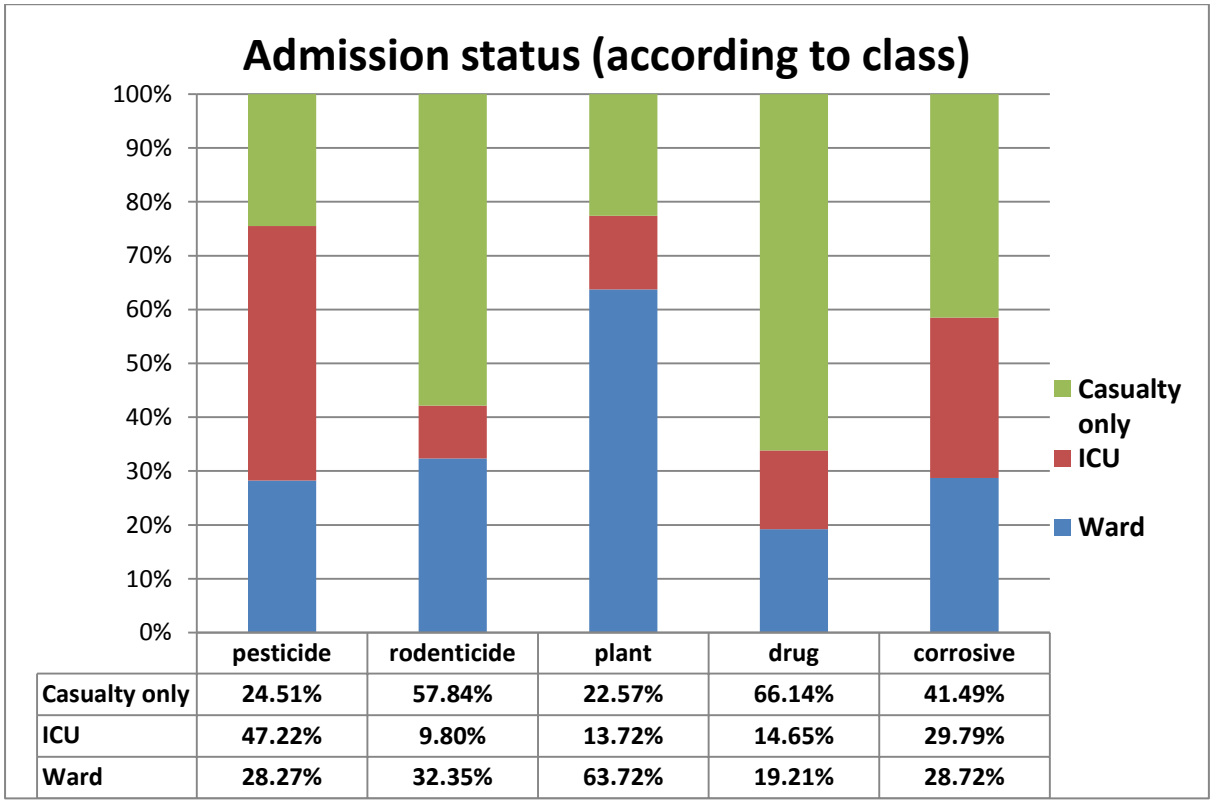


Figure 36: Proportions of different corrosives used, %(n)

OUTCOME OF POISONING AND THE TEMPORAL PROFILE

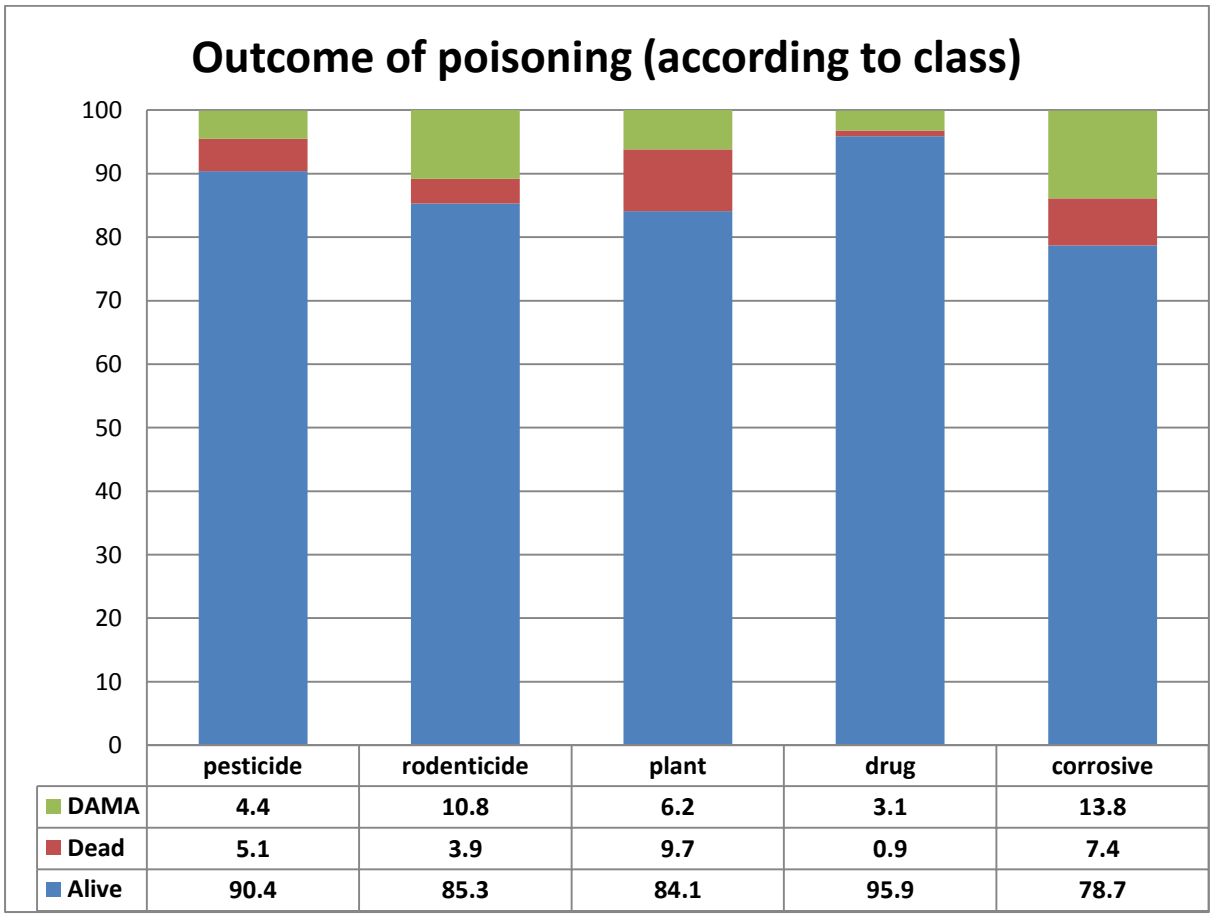
907 out of 2323 patients with poisoning (39%) did not require admission and were discharged from casualty. Of the remaining patients, 733 (31.6%) required treatment in Intensive Care Unit (ICU). Patients with drug overdoses were more likely to get discharged from casualty with 66.1% not requiring admission to ward. Although the percentages of patients discharged from casualty was high with rodenticide and corrosive poisoning constituting 58% and 44% respectively, this may have been related to the greater number of patients discharged against medical advice. The proportion of patients requiring ICU care for individual poisons was as follows: Pesticide (47.2%), Drug (9.8%), Plant (13.7%), Corrosive (14.6%) and Rodenticide (29.8%). (Figure 37)



	pesticide	rodenticide	plant	drug	corrosive
Casualty only	24.51%	57.84%	22.57%	66.14%	41.49%
ICU	47.22%	9.80%	13.72%	14.65%	29.79%
Ward	28.27%	32.35%	63.72%	19.21%	28.72%

Figure 37: Admission status for different classes of poisons

103 out of 2323 patients died resulting in death rate 4.4%. 116 (5%) patients were discharged against medical advice. Mortality rate was highest for plant poisoning with 9.7% of patients succumbing to their illness. Death rate for pesticide poisoning was 5.1%. The rate of overall poor outcome which includes both death and Discharge Against Medical Advice (DAMA) was highest for corrosive poisoning at 21.2% followed by plant poisoning at 15.9%. Rodenticide poisoning was associated with poor outcome in 14.7% of cases. Drug overdose resulted in death in 0.9% of cases. (Figure 38)



	pesticide	rodenticide	plant	drug	corrosive
DAMA	4.4	10.8	6.2	3.1	13.8
Dead	5.1	3.9	9.7	0.9	7.4
Alive	90.4	85.3	84.1	95.9	78.7

Figure 38: Outcomes of different classes of poisons

On analysing the temporal profile of mortality rates, the rates of poor outcome in pesticide poisoning was on a downward trend and reduced from 15% in 2009-10 to 4.5% in 2013-14. There was an increase in the poor outcome rates in rodenticide poisoning rising from 11.2% to 23.8% during the same periods. Although plant poisoning was associated with poor outcome in 24% of cases in 2009-10, it was stable in the next 4 years at 13.5%. (Figure 39)

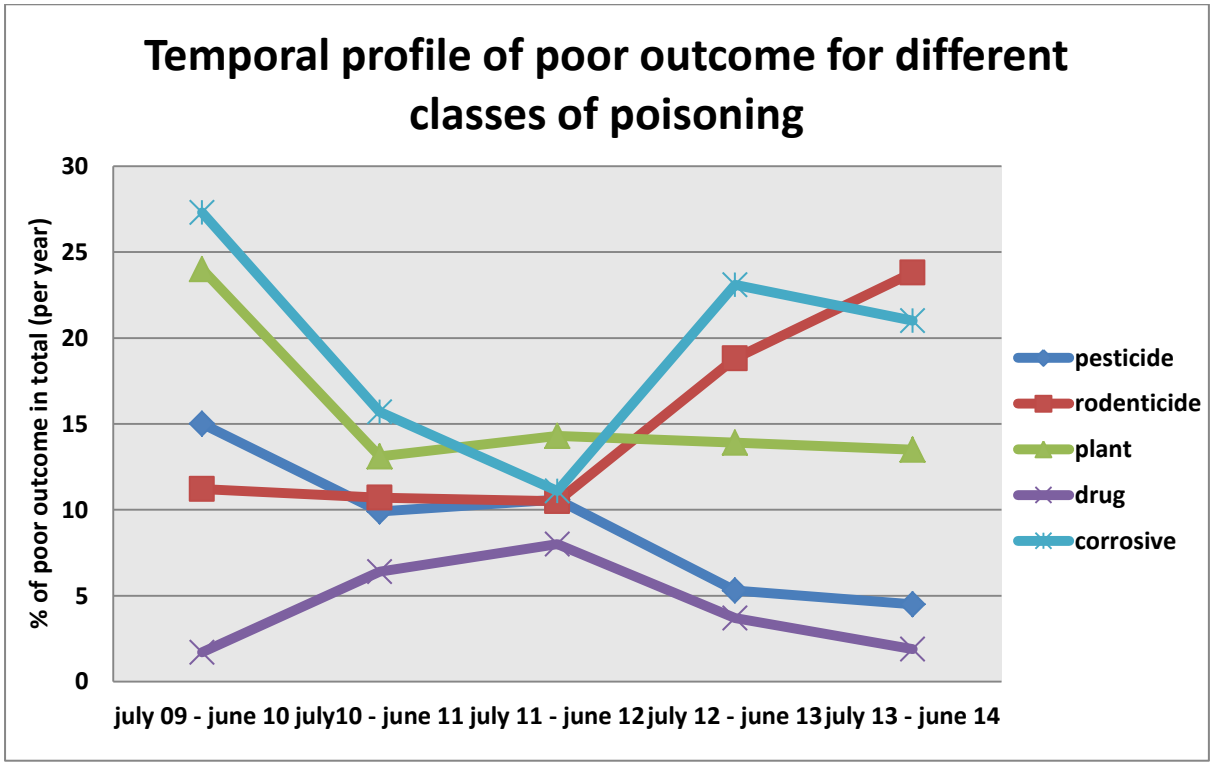


Figure 39: Temporal profile of poor outcome (as % of poisoning) for different classes of poisons

PESTICIDE POISONING

Among the pesticide poisons, 65% and 60% of organophosphorus and organochlorine compound poisoning required ICU care. Pyrethroid poisoning without combination with OP compound was associated with ICU care in 15% of cases and 64% were discharged from casualty. In contrast, Pyrethroid combinations with OP

required ICU admission in 50% of cases. Class Ia and Ib compounds based on the WHO class of pesticide toxicity required ICU admission in 65% and 68% of cases respectively compared to 43% and 44% for class II and class III compounds respectively. Rates of discharge from casualty were also higher in class II and class II compounds. (Figure 40)

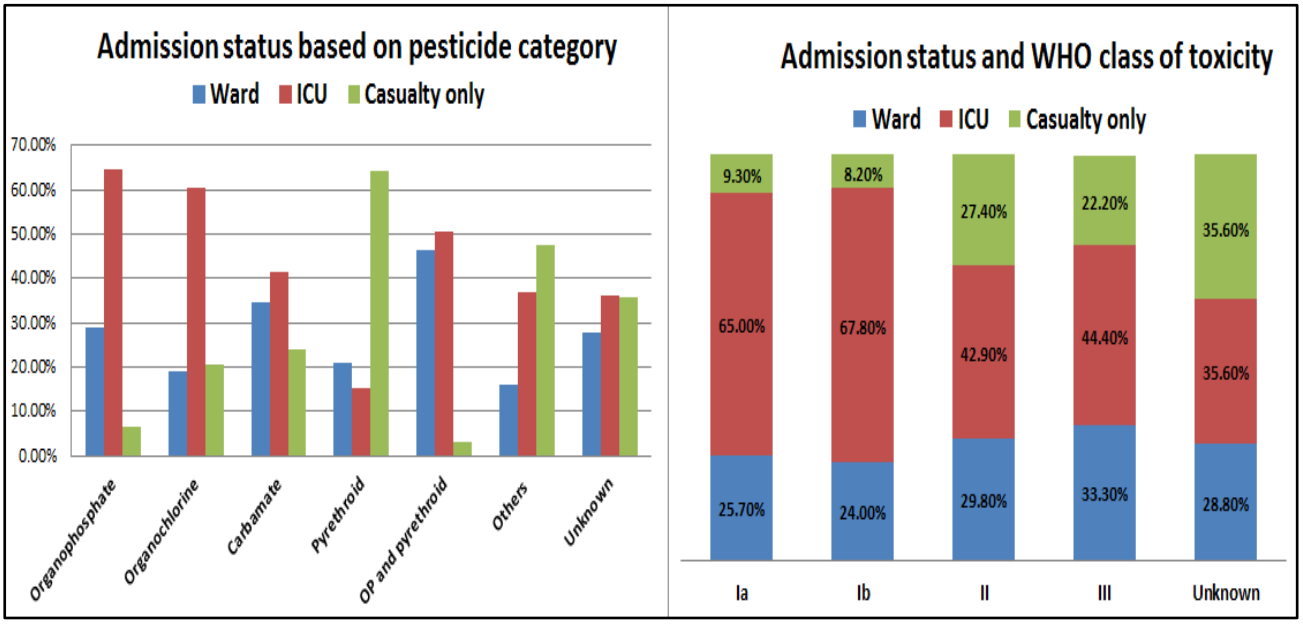


Figure 40: Admission status for different categories of pesticide poisons and according to WHO class of toxicity

The mortality rate was highest for organochlorines among the pesticides at 15.5% with endosulfan poisoning being associated with death in 20.5% of cases. Organophosphorus compounds which constitute the majority of pesticide poisonings had a mortality rate of 5.2% with overall poor outcome in 8.9% of cases. While pure pyrethroid poisoning was not associated with any deaths, formulations of pyrethroid combined with organophosphates had a death rate of 9.3%. On analysing the mortality rates among the different pesticides according to their WHO class of toxicity, it was

found that the highest mortality rate was for the class II compounds at 6% with class Ia and Ib compounds having rates of 5% and 3.3% respectively. However, the overall poor outcome was almost comparable between the three classes at 7.9%, 9.3% and 8.6% respectively for class Ia, Ib and II compounds. Class III compounds were not associated with any deaths. (Figure 41)

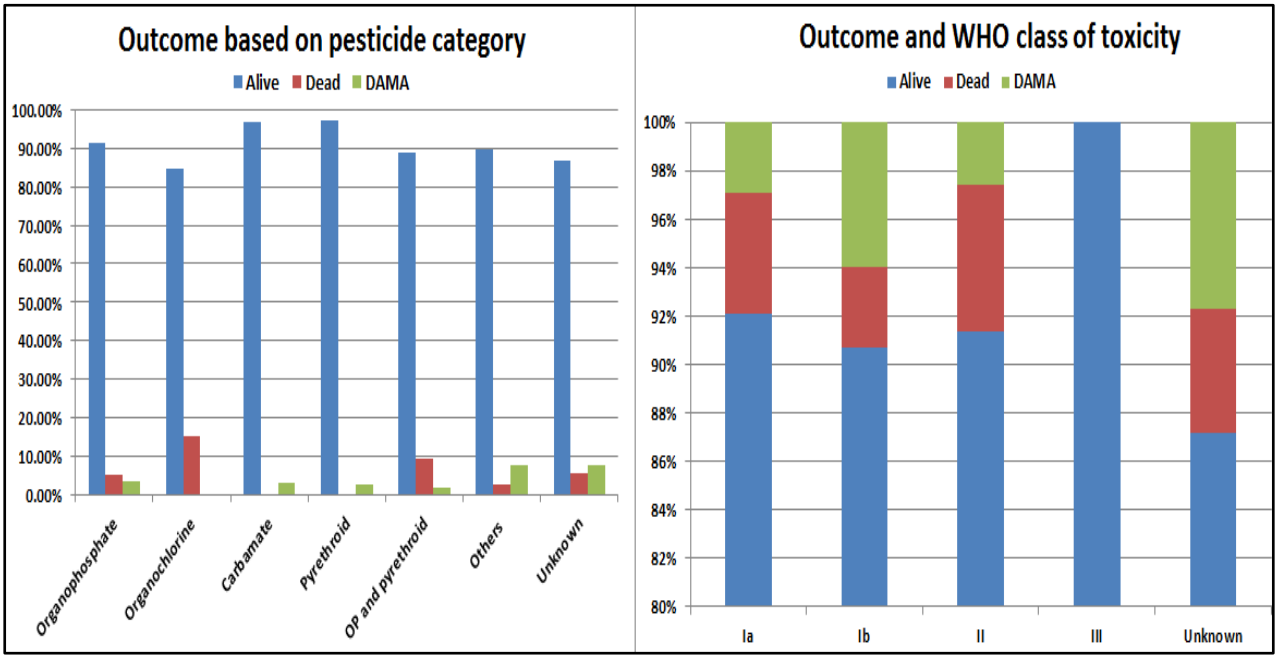
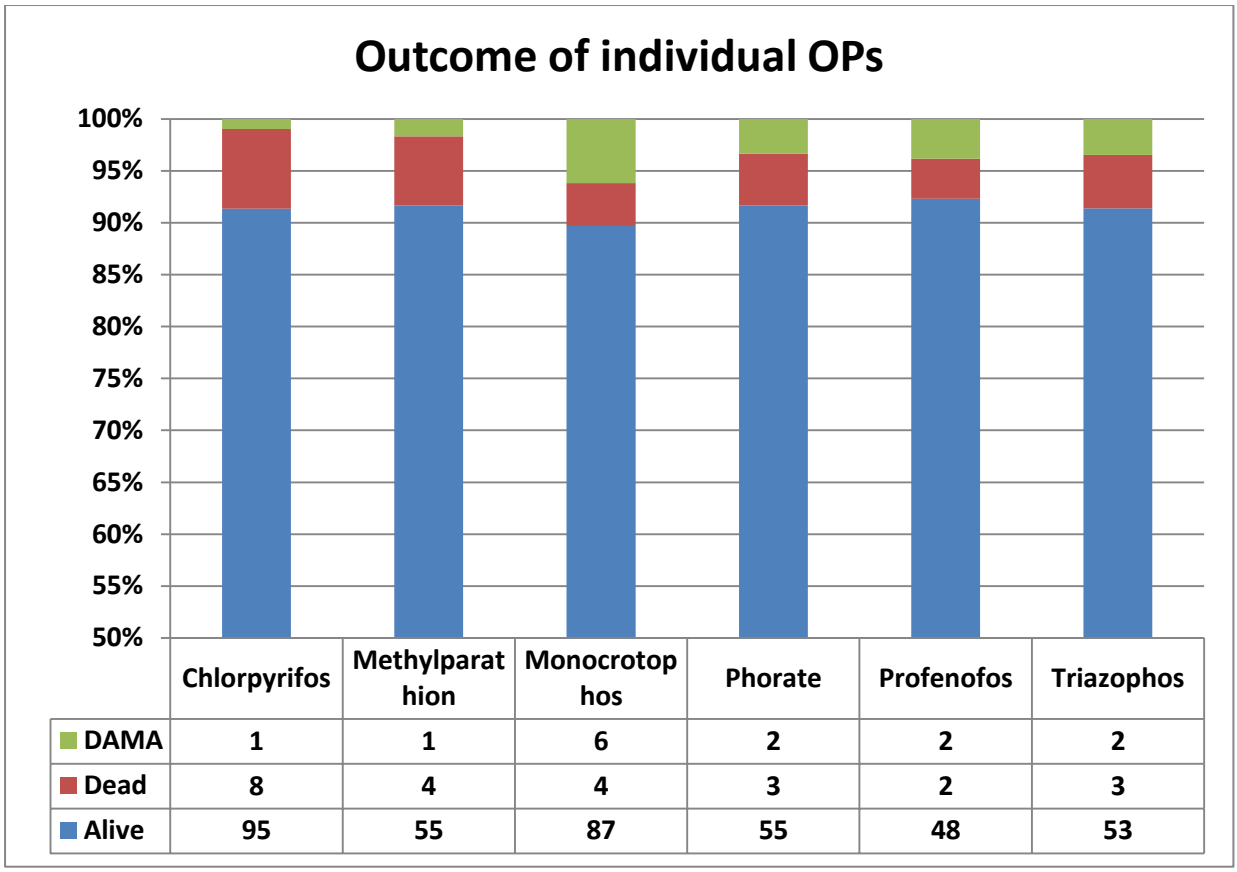


Figure 41: Outcome for different categories of pesticide poisons and according to WHO class of toxicity

Among the individual OP compounds, the highest mortality rate was for Chlorpyrifos at 7.6% but the poor outcomes were greater with monocrotophos at 11.1% as depicted below in Figure 42. The rates of poor outcome for individual OP compounds (with more than 50 cases during the study period) were as follows: Chlorpyrifos (8.6%), Methylparathion (8.3%), Monocrotophos (11.1%), Phorate (8.3%), Profenofos (7.7%), and Triazophos (8.6%).

6 out of the 8 deaths in the chlorpyrifos group occurred in those who had taken a combined formulation with cypermethrin. All 3 deaths in the triazophos group occurred in the combined formulations with deltamethrin.



	Chlorpyrifos	Methylparathion	Monocrotophos	Phorate	Profenofos	Triazophos
DAMA	1	1	6	2	2	2
Dead	8	4	4	3	2	3
Alive	95	55	87	55	48	53

Figure 42: Outcome of individual OP compounds

RODENTICIDE POISONING

Among the rodenticide poisons, the worst outcomes were with yellow phosphorous with death or DAMA occurring in 44.4% of the patients compared to 16.7% and 15.8% in the aluminium phosphide and zinc phosphide groups respectively. Coumarin derivatives or super-warfarins were associated with good outcomes in all cases. (Figure 43)

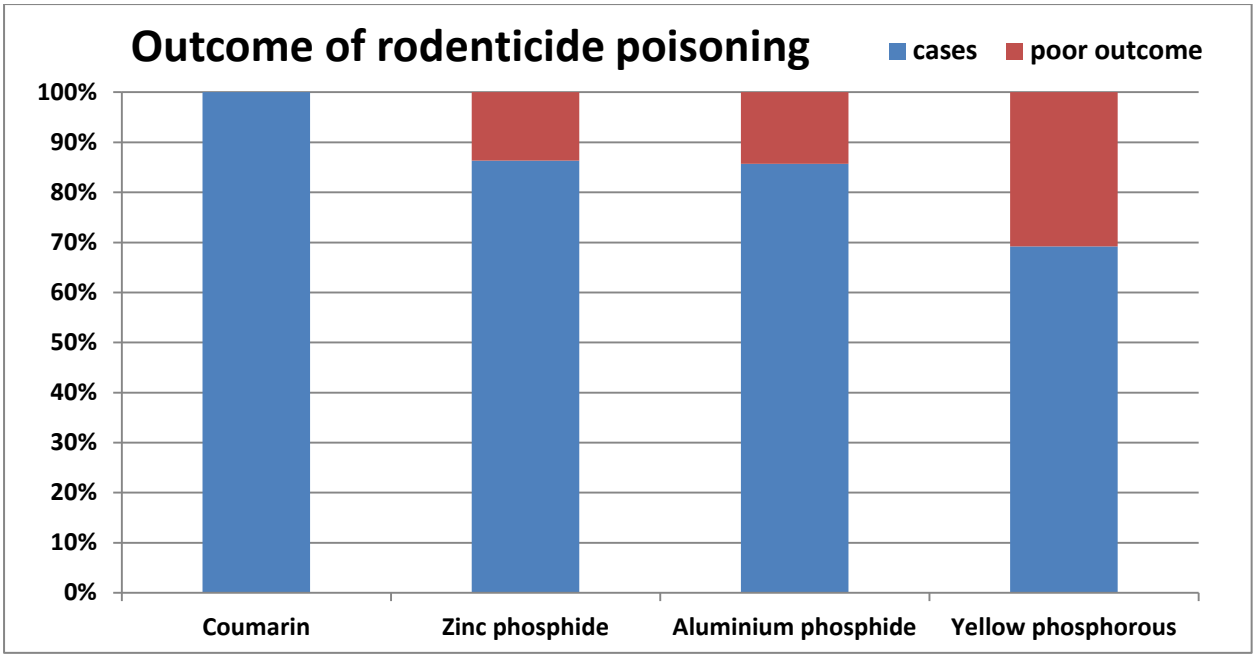


Figure 43: Outcomes of different classes of rodenticide poisoning

PLANT POISONING

Overall rate of poor outcome in plant poisoning was 15.9% with death in 9.7% of cases. However, death rate was significantly higher in the Oduvanthalai group at 19.2% compared to 1.6% for oleander poisoning. Poor outcome in Oduvanthalai poisoning was 27.9% compared to 5.7% for oleander poisoning. (Figure 44)

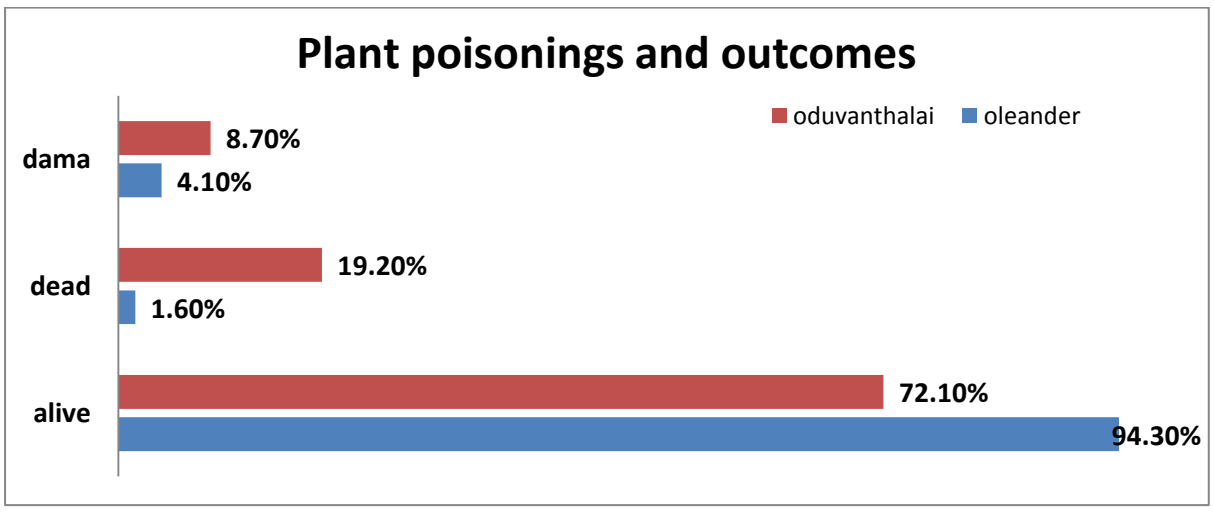


Figure 44: Outcomes of plant poisons - oleander and oduvanthalai

DRUG OVERDOSE

Drug overdose was associated with discharge from casualty itself in 66.1% of cases. Among the individual drugs, ICU admission rates were highest for barbiturates (52.9%) followed by antidepressants (32%). (Figure 45) There were 6 deaths in the drug overdose group with one each due to iron and chromium poisoning. Barbiturates were associated with 1, other antiepileptics with 3 and atypical antipsychotics with 2 deaths.

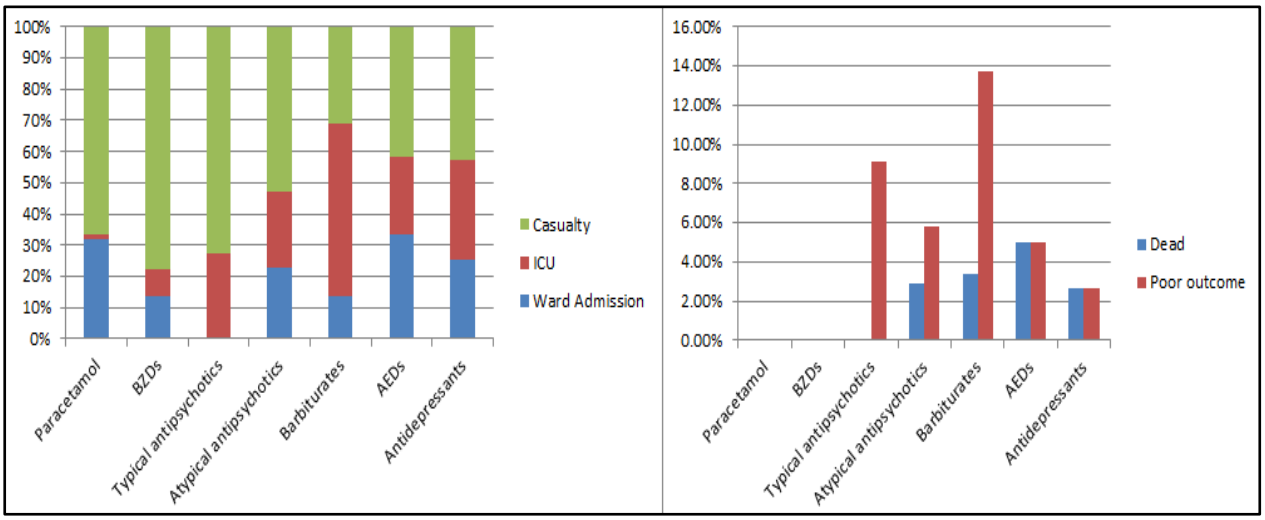


Figure 45: Admission status and outcomes of different groups of drugs

CORROSIVE POISONING

Out of the 7 deaths in the corrosive poisoning group, 2 were due to super vasmol poisoning and 2 were due to toilet cleaning hydrochloric acid. Both the deaths in the super vasmol group occurred in the 2009-10 time period. The super vasmol mortality fell from 11.8% in the first two years of the study to 0 in last two years of the study. 73.1% of super vasmol poisoning patients required ICU admission.

DISCUSSION – (A) TRIPLE CHOLINESTERASE TEST IN ORGANOPHOSPHORUS POISONING

The main findings of this study to evaluate the triple cholinesterase test in OP poisoning were as follows:

TEMPORAL PROFILE OF AChE

RBC-AChE levels were shown to have correlation with clinical presentation and outcomes. Those with severely inhibited AChE at baseline were more likely to have had a late presentation with more severe manifestations. They were also more likely to require mechanical ventilation and develop intermediate syndrome with longer durations of ventilation and intermediate syndrome. Their atropine requirement was also found to be higher. This reflects the utility of RBC-AChE as a good index of severity and prognostic marker in view of its good correlation to clinical markers of severity and complications. This finding supports existing literature on RBC-AChE which suggests that it is a better marker for determining clinical severity than BChE.(82–84)

REACTIVATION STUDIES

DEMONSTRATION OF REACTIVATION

The literature on the efficacy of oximes has been controversial so far. Despite theoretical benefit, definite efficacy has not been proven in clinical settings. This study has biochemically demonstrated that oximes can reactivate the inhibited AChE

enzyme in-vitro. Among the 22 patients with inhibited AChE levels, obidoxime was able to reactivate the enzyme in 13 patients (60%) of the patients.

CLINICAL CORRELATES

Several recent studies have postulated that there may be certain clinical sub-groups in which oxime therapy may be effective.(42,69) The first question that this study sought to answer was to identify the clinical characteristics of the group that may benefit from oxime therapy. **The study shows that the patients who demonstrate in-vitro oxime reactivation of AChE are: (1) those who present within 6 hours (up to 10 hours) after consumption of the compound and (2) those with Diethyl OP compound poisoning.**

Patients who present later have progression ageing of enzyme with time, thereby reducing its reactivation potential.(2,4) This study particularly found significance in the in vitro reactivation of AChE in diethyl compound poisoning with triazophos and chlorpyrifos. This is explained by the differences in ageing kinetics between diethyl and dimethyl compounds as their ageing half-lives are 31 hours and 3.7 hours respectively. This means that at the end of 5 days, the enzyme activity would be reduced to 6.3% among the diethyl compounds whereas the same level would be reached within 16 hours for dimethyl compounds. The dose of oximes required to reactivate the enzyme has also been found to be different between the two groups.(2,46) There were a few patients with dimethyl compound poisoning also in the reactivated group. Patients with dimethyl poisoning with significant reactivation could be identified by using this triple cholinesterase test. **The second question that this study sought to answer was the duration for which the AChE could be**

reactivated. Among the patients with significant reactivation on day 1, oximes could reactivate the enzyme for an average of 2.3 days and in some patients, up to 4 days.

IMPLICATIONS OF THE STUDY

There was a lack of clinical benefit from oxime therapy in several previous studies which has been attributed to the uneven distribution of baseline characteristics including compound type, quantity and variation in time to presentation.(74) Based on the recommendation of *Eddleston* et al to identify sub-groups who would benefit from oxime therapy, the triple cholinesterase test was evolved. It has been advised to administer oximes based on the results of this test.(7,8) The present study is an ex-vivo study that attempts to correlate the laboratory results of oxime-induced enzyme reactivation with the clinical characteristics of the patients. It was found that the patients with significant reactivation and therefore, most likely to benefit from oximes are those who present early after OP poisoning with a diethyl compound.

The triple cholinesterase test can be used to identify patients with significant reactivation of AChE at baseline and serially. These patients would be targets for oxime therapy in clinical settings. The oxime dose can be further titrated to achieve complete reactivation of the enzyme which can be monitored using this test. Oxime should also be continued in-vivo until there is no further significant reactivation of the enzyme using this test. The ability to identify patients who could potentially benefit from oxime therapy through a laboratory test, paves the way for a future tightly controlled clinical trial in which oximes will be administered based on the results of this test with further dosing guided by serial monitoring of cholinesterase status.

INHIBITORY ACTIVITY BIOASSAY

The inhibitory activity bioassay is a functional marker of the OP compound in the patient's sample. The degree of inhibition found in this study was lesser than that found in other studies using this assay.(7,8,88) In our study, it was found that those patients whose plasma inhibited the control AChE to < 90% were associated with poorer clinical outcomes in terms of severity at presentation, need for mechanical ventilation, duration of intermediate syndrome and hospitalisation and the dose and duration of atropine required. The significance of this 10% level of inhibition in the severely poisoned patients is not known and this test requires greater critical examination both in the laboratory and in clinical studies.

DISCUSSION – (B) STUDY OF POISONING PROFILE USING THE POISON DATABASE

Poisoning, especially pesticide poisoning is a common mode of suicide in India.(11) In view of the under-reporting of poisoning cases in India, the WHO advocated for the establishment of poison information or control centres across the country.(17,32) In the department of Medicine in CMC, Vellore, we have a poisoning documentation system in which a dedicated staff prospectively collects information of poisoning cases using a standardised form. This study was done on the poison database which had been compiled between July 2009 and June 2014 over a period of five years.

DEMOGRAPHIC CHARACTERISTICS

There was an overall slight male predominance for all poisonings. But males were more likely to consume pesticide compounds whereas women were more likely to used drugs for poisoning. As in previous studies(13), most of the cases of poisoning occurred in the young working population with over 75% of all poisonings, including the pesticide poisonings, occurring in persons below the age of 40. Most of the patients were farmers or labourers with pesticides being the most commonly used poison. Housewives and students were another large population group accounting for the poisoning cases and these groups were more likely to consume drugs or pesticides. The demographic characteristics are useful to identify the target population for preventive measures for poisoning and suicide which should include young workers from the farming community, students and housewives.

COMPOUNDS USED FOR POISONING AND THEIR TIME-TRENDS

Most studies in India have found pesticides, especially organophosphate compounds, to be the most commonly used agents for poisoning.(1,18) This was reflected in our study as well with half the cases being due to pesticide poisoning. Drugs accounted for a quarter of the cases with plant poisoning in one in every tenth person presenting with poisoning. What makes these pesticides the most popular compounds for poisoning is their easy availability and access, especially in a farming community.

DECLINE IN POISONING CASES AND POSSIBLE REASONS

It was noted from this study that there was a sharp decline in the overall numbers of all poisonings from 2010-11 to 2011-12 and the numbers remained stable thereafter. As this fall occurred across all classes of poisoning with no change in the proportion of different compound classes, it does not appear to be a reflection of a change in epidemiology of attempted suicide (Deliberate self-harm or DSH) and poisonings at a community level. There could be several reasons for this decrease in numbers of all poisoning cases as detailed below:

1. There may have been a fall in the DSH rates among the community during this time period. This is unlikely as there has been no change in the social circumstance and no apparent similar national or regional trend that has been documented.
2. There could be change in the access to specific compounds that would influence compound-specific DSH rates. This decline has affected all compounds and therefore unlikely to be due to change in access to poisons.

3. The apparent decline may represent a change in the referral pattern at the district level. The ICU facilities in the Government Medical College in the district have been upgraded and there has been strengthening of taluk hospitals which are handling poisoning cases. There are other private hospitals with ICUs in the district. The upgrading of the tertiary and secondary level hospitals particularly in the government health system and also in the private health system may have led to the decrease in the poisoning referrals to this centre which for long had been the only centre with ICU facilities for poisoning management in the district. Hence to improve poisoning care at the district level, we need to focus across hospitals to improve guidelines, training and inter-hospital referral.

COMPOUNDS WITH AN INCREASING TREND

Although there was a downward trend in the numbers of all poisonings, there were certain classes of compounds with an increasing trend. This was especially seen among the combined formulations of OP with pyrethroids which accounted for 8% of pesticide poisonings although there was a decline in the proportions of pure pyrethroids. This suggests that there are increasing marketing, sales and use of OP/pyrethroid combination formulations which is leading to the rising trend. The rising trend and higher mortality suggests the need for restriction of this class of compounds. The relative fall in numbers was less among the pesticides than other classes of compounds. Chlorpyrifos and monocrotophos were the most popular OPs and the numbers of the individual OP compounds remained stable post-2011.

COMPOUNDS WITH A DECREASING TREND

The decline in numbers was more prominent among certain classes of compounds. Pure pyrethroids, which had good outcomes, showed a drop in

proportions compared to the OP and pyrethroid combination compounds which are associated with poor prognosis. Organochlorines which have a high mortality rate were also on progressive downward trend. This may be due to the fall in numbers of endosulfan poisoning. There was also a sharper drop in proportions of all the corrosives including super vasmol hair dye.

EFFECT OF ENDOSULFAN RESTRICTION?

A study done in Sri Lanka by *Eddleston et al* showed that there was a fall in poisoning mortality without fall in numbers after restriction of the most toxic pesticides including endosulfan and the WHO class I toxicity compounds.(24) In India, the Supreme court passed an order banning endosulfan in May 2011.(90) Although the ban could not be strictly implemented, measures to restrict endosulfan use were instituted and it was planned to phase out endosulfan by 2017 in the country. In our study, it was found that endosulfan, which had a high mortality rate of 20%, was on a sharp decreasing trend and that there were no cases of endosulfan poisoning after June 2013. This could reflect the effectiveness of the restriction measures and strengthens the argument that restriction or ban of the highly toxic compounds, including WHO class Ia and Ib compounds, could eventually reduce poisoning mortality. In this study, there were more than 500 cases of OP poisoning, 70% of which were due to the Class I and Class II OP compounds. Nearly all the 25 deaths due to OP compounds could have been prevented had these compounds been restricted as has been done in Sri Lanka.

OUTCOMES OF POISONING

Although poisoning mortality, including mortality due to pesticides, in this study was 5% which was lower than that found in other studies in India, there were certain compounds associated with poorer outcome than others.(18) While OP compounds had 5% mortality rate and pure pyrethroids were not associated with any deaths, the combined formulations of OP and pyrethroids had a much higher mortality rate at 9.3%. This could be due to the synergistic effect of the two classes of compounds or it could reflect the effect of the higher dose of the OP compound in these formulations. This finding of increased toxicity in this group is similar to another study done in the same institution.(91) The ICU admission rates and mortality rates were uniformly high in WHO class Ia, Ib and II compounds reflecting their highly toxic nature compared to the WHO class III compounds. These findings should be taken note of to implement further preventive and restrictive measures.

Super vasmol poisoning was associated with 2 deaths, both of which occurred in the first year of the study period. The reduction in mortality rates following this period may be related to the reduction in the concentration of the toxic component, PPD (ParaPhenylene Diamine) in the commercial form of this hair dye.(92,93) In view of high mortality due to Super vasmol poisoning, our institution had worked with the company to reduce the PPD concentration. This reduction may have led to the reduced mortality. This finding also emphasises the importance of clinicians working with chemical companies towards reformulation to reduce toxicity.

Other compound classes associated with poor outcomes were the corrosives and rodenticides as well as oduvanthalai. These compounds not only had high

mortality rates, they also had high rates of discharge against medical advice which may be a reflection of the poor prognosis of these patients. While these poisonings are difficult to treatment, the analysis suggest that they should be the target for focussed improvement in management through training, guidelines and research into specific treatment strategies to improve outcomes.

PREVENTIVE MEASURES

It appears from this study that the implementation of restriction on endosulfan helped in reducing the numbers of this toxic compound. It may be prudent to consider implementation of similar measures for the other highly toxic pesticides of WHO class I and II to curb their use and reduce poisoning mortality. The specific characteristics of the combined formulations of OP with pyrethroids also need to be investigated in view of high mortality rates.

The study showed the benefit of a prospective data base in monitoring trends of poisoning which shows the impact of change in referral patterns, pesticide restriction (endosulfan), compound reformulation (Super vasmol), marketing of new formulations (OP with pyrethroid combined formulations) and identifying high risk groups for improvement in management (corrosives, oduvanthalai and rodenticides). Such data need to be fed back to the Government to influence pesticide policies. It also shows the need for establishment of Poison centres in each medical college. Audit of outcomes of poisonings can identify priorities for improvement in poison management through clinical guidelines and training and also research priorities for strategies to reduce mortality and poor outcome.

CONCLUSION

Organophosphorus poisoning is a major problem in South India. Oximes, which are the specific antidotes for OP poisoning, have not shown clear benefit in clinical studies and recommendations regarding their use is controversial. This study has shown that oximes can induce ex-vivo reactivation of the inhibited acetylcholinesterase enzyme to adequate levels in at least 60% of patients. The patients in whom the enzyme was reactivated with oximes were those who presented early to the hospital, within 6 hours (± 4 hours) and those who had consumed the diethyl OP compounds, such as triazophos and chlorpyrifos. Among the patients with reactivation, oximes continued to reactivate the enzyme significantly for an average of 2.35 days (± 1.7 days).

It has been shown from this study that this test can be applied in a clinical setting to identify patients with good reactivation at admission. Therefore this test has potential use to plan administration of oximes in these patients who are most likely to benefit from oxime therapy. This test can also be used temporally on serial samples to guide oxime dose and duration of administration. This test could be used for a future controlled clinical trial to test the efficacy of selective administration of oximes in patients who demonstrate ex-vivo oxime reactivation.

Although serum from patients with severe poisoning had greater inhibitory activity, the role of the inhibitory assay as a functional indicator of the presence of the OP compound in the plasma is not yet clear.

The second part of the study which dealt with the profile of poisonings has shown the benefit of maintaining a poison database in order to identify the toxic risks in the community. Certain time-trends have become apparent from this study including the sharp decline in all poisonings after 2011 which may be due to a change in the referral pattern to this institution. The efficacy of restriction measures was reflected in the fall in numbers and mortality of the organochlorines, especially endosulfan. Further compounds have been identified for restriction including the class I and II pesticides and OP/pyrethroid combinations in view of their high rates of poor outcomes. The effect of reformulation has been demonstrated by the fall in mortality due to super vasmol and increased mortality with OP and pyrethroid combined formulations. High-risk poisoning groups with poor outcomes have been identified which include corrosives, rodenticides (mainly yellow phosphorus) and Oduvanthalai. Training guidelines and improvement in management strategies need to be put in place to improve outcomes in these poisonings. The results from this database can used to assist in framing public health policy regarding pesticide use and restriction. There is need to establish a poison control centre in each medical college to monitor the poisoning risks at a regional level.

LIMITATIONS

(A) TRIPLE CHOLINESTERASE TEST IN ORGANOPHOSPHORUS POISONING

1. The sample size was small as this was a pilot study and even among these patients, there were a few patients who did not show significant baseline inhibition of AChE despite toxidrome features being present.
2. The inhibitory bioassay did not show very low levels and most values were above 80%. This may be related to the dilutions used. The numbers tested was also small. Further studies would be required to determine the significance of this test.
3. HPLC measurement of OP compounds in the patient's blood could not be done to support the clinical and laboratory studies.
4. This was a clinico-laboratory based study and only shows the reactivation of the AChE enzyme by obidoxime ex-vivo on blood samples taken from the patient. Further controlled clinical trials are required where the triple cholinesterase test is used to guide oxime therapy in selected cases of OP poisoning.

(B) STUDY OF POISONING PROFILE USING THE POISON DATABASE

One limitation from this study was the large number of unknown compounds which could not be analysed. This was a limited retrospective study to determine the changing profile and outcomes of poisoning and further detailed analysis including clinical characteristics for each different poison can be derived from the poisoning database for later studies.

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ANNEXURES

ANNEXURE 1 – PATIENT INFORMATION SHEET

Study Title: A STUDY TO EVALUATE THE USE OF TRIPLE CHOLINESTERASE TEST IN PATIENTS WITH ORGANOPHOSPHORUS POISONING

Subject's Name: _____

Date of Birth / Age: _____

Your family member has been admitted with pesticide poisoning due to Organophosphorus insecticides. The treatment of this condition consists of supportive measures such as drugs to neutralise the effect of poison, close monitoring and supports for respiration where required. These poisons act by blocking proteins called enzymes in the brain and nerves causing paralysis and breathing difficulty. The name of this protein that is blocked is called cholinesterase. There are a class of drugs called oximes that can reverse the blocking of cholinesterase by the pesticides. However it is not clear from research whether oximes are beneficial to patients. Hence oximes are not used for treatment in our hospital. Since this condition is a serious one, there is need for better treatments. In this study we are trying to develop a test to assess whether individual patients may benefit from oxime treatment. This study aims to test the levels of enzyme protein cholinesterase and the pesticide level in the patient's blood and also after adding oxime drug to the blood sample to increase the activity of the enzyme. This will be done on the patient's blood sample in the lab. If we can show that oximes increase the enzyme activity, then there may be a role for oxime treatment in such patients. Hence this test may help future patients in improving their

management, by deciding whether oxime therapy would be useful or not and also in following up these patients.

Participating in this study is purely voluntary and you can decide to withdraw your relative from the study at any time. Your relative will receive all standard treatment according to hospital protocols and your doctor will decide on the optimum treatment for your relative. This is a purely observational study and does not involve any additional drug treatment apart from the standard treatment. This study will not in any way influence the treatment that your relative is receiving in the hospital. All the patient's health records and any other data arising from the study would be kept confidential and used only for scientific purposes.

Inclusion into this study involves signing a consent form and the following:

1. Collection of clinical data including details of poisoning
2. Daily clinical examination (which is a part of routine care)
3. Blood tests – will be performed daily

The procedures of the study not painful and do not cause any threat to health. Please contact me if you have any doubts. (Dr._____, Phone No._____)

ANNEXURE 2 – INFORMED CONSENT FORM

Informed Consent form to participate in the study

Study Title: A STUDY TO EVALUATE THE USE OF TRIPLE CHOLINESTERASE TEST IN PATIENTS WITH ORGANOPHOSPHORUS POISONING

Subject's Name: _____

Date of Birth / Age: _____

Name of relative: **Relationship:**

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions.

(ii) I understand that the participation of my relative in the study is voluntary and that I am free to withdraw this consent at any time, without giving any reason, without my medical care or legal rights being affected.

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my relative's health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw consent from the trial. I agree to this access. However, I understand that my relative's identity will not be revealed in any information released to third parties or published.

(iv) I agree not to restrict the use of my relative's data or results that arise from this study provided such a use is only for scientific purpose(s).

(v) I agree to allow my relative to take part in the above study.

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Representative (Next of kin): _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: ____/_____/_____

Name & Address of the Witness: _____

ANNEXURE 3 – PATIENT PROFORMA

Clinical research form Serial number

Ascertainment criteria for OP poisoning (Tick appropriately):

1. Patients who present with history of pesticide poisoning with an identified OP compound.

2. Patients who present with history of pesticide poisoning with typical toxidrome of organophosphate poisoning, and low BChE levels.

3. Patients who present without a history of pesticide poisoning but with typical toxidrome of OP poisoning and low BChE levels.

Informed consent obtained: Yes/No

DEMOGRAPHIC DETAILS

1. Name:
2. Hospital ID:
3. Age:
4. Sex: 1.Male / 2.Female
5. Marital status: 1.married / 2. Single / 3. Others
6. Education: 1.Uneducated / 2.Educated
7. Profession: 1.Unskilled/2.Semi-skilled/3.Skilled

COMPOUND

1. Compound identified: Yes / no

2. Compound name:
3. Method by which compound identified: Name given by patient/leaflet/bottle brought
4. Type of compound: 1. Dimethyl / 2. Diethyl / 3. Others
5. Percentage of compound :
6. Compound quantity(volume):

DETAILS REGARDING INGESTION

1) Time and date of consumption:
2) Time and date of First medical contact:
3) Time and date of arrival ay CMC:
4) Treatment given outside : 1. Skin decontamination / 2.Induced Emesis / 3. Gastric Lavage/ 4. Atropine (dose) / 5. PAM (dose) / 6.Intubation
5) Toxidrome: 1. Salivation / 2. Lacrimation / 3. Urination / 4. Defecation / 5. Vomiting / 6.Seizures / 7. Breathlessness / 8. Fasciculations / 9.Altered sensorium
6) Severity of poisoning by Namba scale: Mild/Moderate/Severe

CLINICAL FEATURE – ON ADMISSION IN E&D

1. GCS at presentation:
2. Pupils size: PINPOINT / DILATED/ NORMAL (2-5MM)
3. Pulse rate at presentation:
4. Blood pressure at presentation:
5. Respiratory rate at presentation:

6. Saturation at presentation:
7. GRBS
8. Symptoms at presentation:

Vomiting/diarrhoea/abdominal pain/salivation/sweating/ urinary incontinence /drowsiness/breathing difficulty/seizures/bleeding/giddiness/altered sensorium/agitation/blurring of vision/ sedation/asymptomatic

9. Signs at presentation

Diaphoresis

Pupil size

Lung crepitation

Salivation

Frothing at mouth

Fasciculations/muscle weakness

Single breath count

Paradoxical breathing

Abdominal tenderness

Fever

Severity of poisoning (by Namba Scale): Mild/Moderate/Severe

10.Investigation: Laboratory BChE:

12.Serial clinical assessment

	DAY1	2	3	4	5	6	7
Heart rate							
Blood pressure							
GCS							
MUSCLE POWER-							
Shoulder Elbow							
Hand grip							
Hip							
Neck Muscle weakness-							
neck holding time Respiratory muscle weakness Tidal volume							

Pressure support PEEP							
Single breath count							
Miosis							
Crepitation							
Salivation							
Diarrhoea							
Cholinergic crises present/absent							
Atropine bolus dose given (mg)							
Atropine infusion rate (ml/hour * hours)							

Total atropine dose (mg)							
Sedation present/absent							

Intermediate syndrome: Present/absent

Duration of intermediate syndrome:

13. Did the patient Required ICU admission

14.Date of admission date of discharge

15.Did the patient require ventilator support?

16.Date intubated Time intubated

17.Indication for mechanical ventilation

18.Duration of mechanical ventilation

19.Did the patient require tracheostomy yes no

20.Duration of ICU stay.

21.Complications

- Cardiac arrest-yes/no
- Respiratory arrest-yes/ no

- Infective complications-yes/ no
    - What infection:
    - Criteria for diagnosis of infection:
    - Chest x-ray
    - Cultures:
- Atropine delirium: Yes/No
    - Duration:

22. Treatment:

Gastric lavage at CMC: Yes/No

Charcoal at CMC: Yes/No

Atropine: Yes/No Duration (in days):

Total dose of atropine:

23.Death – yes/ no

24. Cause of death:

Cholinergic crises

Ventilatory and airway problems:

Infections:

Others:

Summary data:

OP compound: ----------/ Unknown Concentration: Volume:

Severity of poisoning: Mild/Moderate/Severe

Cholinergic crises present/absent Duration of cholinergic crises:

Atropine duration: Total atropine dose:

GCS at admission:

Duration of low GCS:

Requirement of mechanical ventilation: Yes/No

Duration of mechanical ventilation:

Intermediate syndrome: Yes/No

Duration of intermediate syndrome:

Duration of hospitalisation

Final outcome: Alive/ Dead/ Discharged against medical advise

Laboratory measurements:

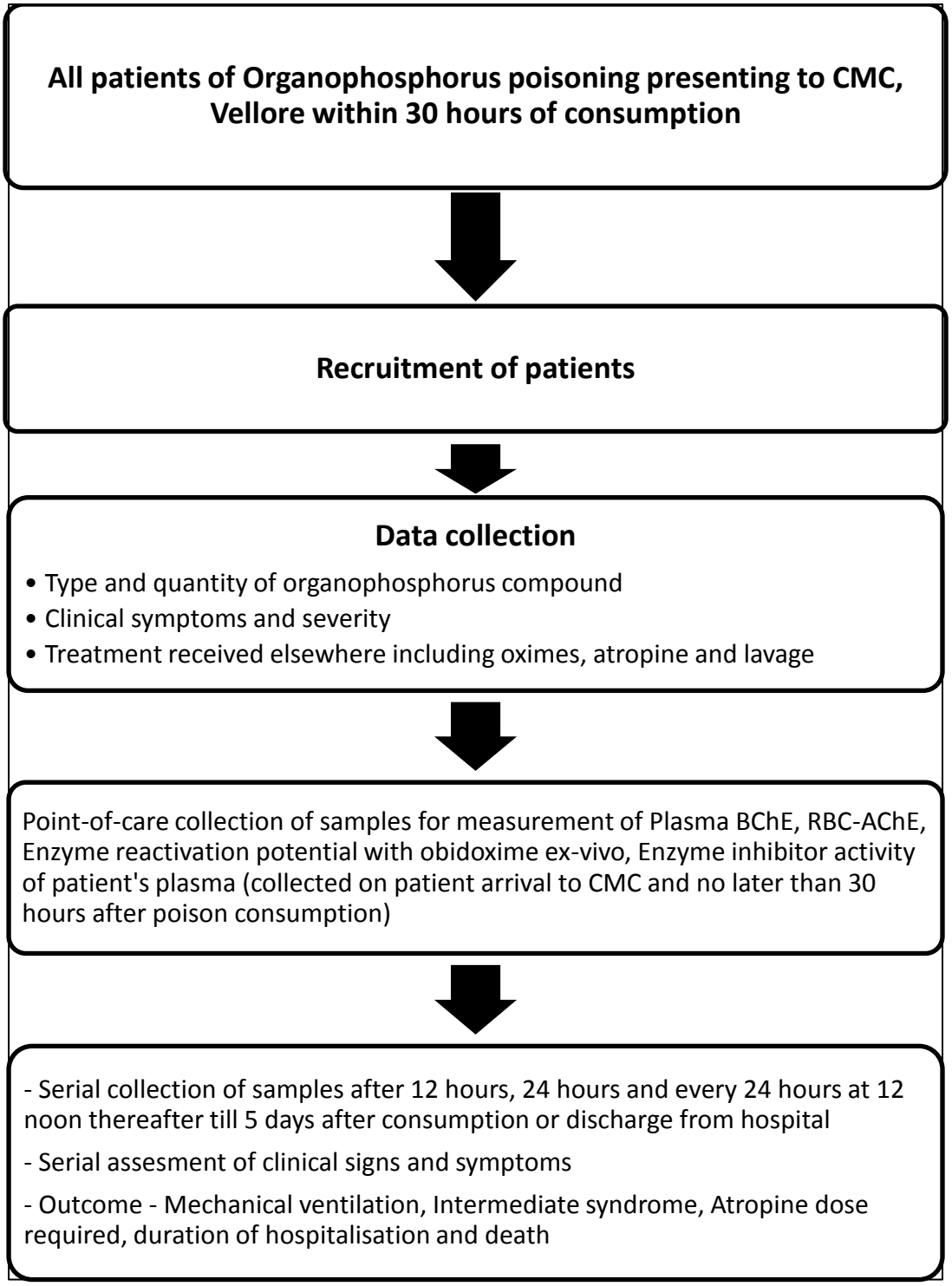
11.Serial monitoring of cholinesterase

	Day1	Day2	Day3	Day4	Day 5

	0	12	24				
BChE(IU/L)							
RBC AChE(U/gmHB)							
Enzyme reactivability (U/gmHB)							
Inhibitor activity of patient's plasma(%inhibition)							

Name of person who filled the form and signature

ANNEXURE 4 – DATA AND SAMPLE COLLECTION PROTOCOL



ANNEXURE 5 – STANDARD OPERATING PROTOCOL OF TRIPLE CHOLINESTERASE TEST

MATERIALS REQUIRED FOR THE ASSAYS

1) Phosphate buffer (= PP, 0.1 M, pH 7.4)

Solution 1: Na2HPO4*2H20, MW 177.9 (17.8 g in 1000 ml distilled water)

Solution 2: KH2PO4, MW: 136.1 (13.6 g in 1000 ml distilled water)

800 ml of solution 1 was mixed and adjusted with solution 2 to pH 7.4 with pH-meter and filtrate and stored in amber bottle at +4°C.

2) Diluting reagent for blood dilution: Distilled water

3) 5,5´-dithio-bis(2-nitrobenzoic acid) (= DTNB, Ellman´s reagent)

396.3 mg DTNB (MW 396.3, Sigma) was dissolved in 100 ml of phosphate buffer (0.1 M, pH 7.4) and stored aliquots at ≤-20°C. The concentration is 10 mM in the solution and 0.3 mM in the cuvet.

4) Acetylthiocholine iodide (=ASCh)

41.12 mg ASCh (MW 289.2, Sigma) was dissolved in 5.0 ml distilled water and stored aliquots at ≤-20°C. The concentration is 28.4 mM in the solution and 0.45 mM in the cuvet.

5) S-Butyrylthiocholine iodide (= BuSCh)

200.47 mg BuSCh (MG: 317.2, Sigma) was dissolved in 10.0 ml distilled water and stored in aliquots at ≤-20°C. The concentration is 63.2 mM in the solution and 1.0 mM in the cuvet.

6) Ethopropazine (selective BChE inhibitor)

20.94 mg ethopropazine hydrochloride (MW 348.94, Aldrich) was dissolved in 10 ml of 0.01 M HCl under stirring and mild heating and stored in aliquots at ≤-20°C. The concentration is 6.0 mM in the solution and 0.02 mM in the cuvet.

7) Transformation solution

200 mg K3[Fe(CN)6] (MW 329.2), 50 mg KCN (MW 65,1), and 1000 mg NaHCO3 (MW 84.0) were mixed with 1000 ml distilled water, followed by 0.5 ml Triton X-100 and stored in a tightly sealed amber glass bottle at ambient temperature.

8) Obidoxime solution:

7.54mg of obidoxime dichloride monohydrate(MW 377.2) was dissolved in 1.0ml of distilled water and frozen in small aliquots (50 µl) at -20C at a concentration of 20mM.

9) Diluting reagent for test erythrocytes

100 µl Triton X-100 (Sigma) was added to 100 ml phosphate buffer (0.1 M, pH 7.4) and mixed thoroughly with final concentration of Triton X-100 at 0.1%

10) Test erythrocytes and test plasma

Blood was taken in EDTA tube, centrifuged (10 min, 3000 rpm) and transfer plasma into tube ("test plasma"). The dilute packed erythrocytes with PP were mixed, centrifuged and supernatant was removed. This was repeated three more times. Washed, packed erythrocytes were diluted with 10 volumes diluting reagent for test erythrocytes and mixed. Aliquots of test erythrocytes and test plasma were transferred in separate tubes and stored at ≤-20°C.

11) Cuvets: Polystyrene cuvets (10 x 10 x 45 mm, e.g. VWR 634-0675)

MEASUREMENTS IN THE ASSAYS

a) Haemoglobin content

Sample preparation: 0.3 ml of the blood dilution and 2.7 ml of the transformation solution were mixed in a cuvet and extinction was read after 10 min against a reference cuvet containing only transformation solution.

Conditions: Wavelength: 546 nm, Temperature: Ambient, Extinction coefficient: $10.8 * 10^3 M^{-1} cm^{-1}$, Cuvets with 1 cm light path

Calculation:

$$\mu M\ Hb^* = - \frac{\text{extinction (mA)}}{10.8}$$

* molarity refers to iron

b) Erythrocyte AChE activity

Conditions: Wavelength: 436 nm, Extinction coefficient: 11.28 * 103M-1cm-1, Temperature: 37°C, Cuvet volume: 3.16 ml, Recording time: 2 min, Cuvets with 1 cm light path

Substrate blank%:

- 3000 µl phosphate buffer (PP), 100 µl DTNB, 10 µl ethopropazine, 50 µl ASCh
- PP, DTNB and ethopropazine were added to cuvet, equilibrated for 10 min, ASCh was added and mixed

Sample:

- 2800 µl PP, 100 µl DTNB, 200 µl blood dilution (sample), 10 µl ethopropazine, 50 µl ASCh
- PP, DTNB, ethopropazine and sample were added to cuvet, equilibrated for 10 min, ASCh was added and mixed

Calculation:

$$\mu M/min = \frac{mA/min\ (sample) - mE/min\ (blank)}{11.28}$$

Referred to haemoglobin content:

$$\mu M/min/\mu mol\ Hb = \frac{AChE\ activity\ (\mu M/min) * 2\$}{\mu M\ Hb}$$

$ Factor for correction of different dilution in the determination of haemoglobin and AChE since the whole blood specimen has been diluted with 0.1ml of sample to 2.0ml with diluting reagent

c) Reactivability

Sample preparation:

5 µl of Obidoxime solution(20mM) was added to 1.0ml of phosphate buffer or 1.0ml of patient blood dilution, incubated for 30min at 37°C

Conditions: Wavelength: 436 nm, Extinction coefficient: 11.28 * 103M-1cm-1, Temperature: 37°C, Cuvet volume: 3.16 ml, Recording time: 2 min, Cuvets with 1 cm light path

Substrate blank%:

- 2500 µl phosphate buffer (PP), 100 µl DTNB, 10 µl ethopropazine, 500 µl incubate, 50 µl ASCh
- PP, DTNB and ethopropazine were added and incubated in cuvet, equilibrated for 10 min, ASCh was added and mixed

Sample:

- 2500 µl PP, 100 µl DTNB, 500 µl incubate, 10 µl ethopropazine, 50 µl ASCh
- PP, DTNB, ethopropazine and sample were added to cuvet, equilibrated for 10 min, ASCh was added and mixed

Calculation:

$$\mu M/min = \frac{mA/min\ (sample) - mE/min\ (blank)}{11.28}$$

Referred to haemoglobin content:

$$\mu M/min\ /\mu mol\ Hb = \frac{AChE\ activity\ (\mu M/min) * 2\$}{\mu M\ Hb}$$

$ Factor for correction of different dilution in the determination of haemoglobin and AChE since the whole blood specimen has been diluted with 0.1ml of sample to 2.0ml with diluting reagent

d) Plasma-BChE activity:

Sample preparation:

Thawed plasma was centrifuged with high speed for 1-2 min to remove cryoprecipitate.

Conditions: Wavelength: 436 nm, Extinction coefficient: 11.28 * 103M-1cm-1, Temperature: 37°C, Cuvet volume: 3.16 ml, Recording time: 2 min, Cuvets with 1 cm light path

Substrate blank%:

- 3000 µl phosphate buffer (PP), 100 µl DTNB, 50 µl BuSCh
- PP, DTNB and ethopropazine were added to cuvet, equilibrated for 10 min(37°C) and reaction with BuSCh was started

Sample:

- 3000 µl PP, 100 µl DTNB, 10 µl sample(Plasma), 50 µl BuSCh
- PP, DTNB, ethopropazine and sample were added to cuvet, equilibrated for 10 min(37°C) reaction with BuSCh was started

Calculation:

$$\mu M/min = \frac{mE/min\ (sample) - mE/min\ (blank)}{11.28}$$

Referred to plasma content:

mU/ml plasma= µM/min * dilution factor (=316)

e) Inhibitory activity

Sample preparation:

Thawed plasma was centrifuged with high-speed for 1-2min to remove cryoprecipitate. 30 µl of patient plasma was added to 100 µl test erythrocytes and incubated for 60 min at 37°C. Additional control was prepared by adding 30 µl test plasma to 100 µl test erythrocytes and incubating for 60 min at 37°C.

Conditions: Wavelength: 436 nm, Extinction coefficient: 11.28 * 103M-1cm-1, Temperature: 37°C, Cuvet volume: 3.16 ml, Recording time: 2 min, Cuvets with 1 cm light path

Substrate blank%:

- 3000 µl phosphate buffer (PP), 100 µl DTNB, 10 µl ethopropazine, 50 µl ASCh
- PP, DTNB and ethopropazine were added to and incubated in cuvet, equilibrated for 10 min(37°C) and mixed with ASCh

Sample:

- 3000 µl PP, 100 µl DTNB, 10 µl ethopropazine, 50 µl incubate, 50 µl ASCh

- PP, DTNB, ethopropazine and sample were added to cuvet, equilibrated for 10 min(37°C) and mixed with ASCh

Calculation:

$$\mu M/min = \frac{mE/min\ (sample) - mE/min\ (blank)}{11.28}$$

Calculation of inhibitory activity:

$$\%\ Inhibition = \frac{Activity\ incubate\ (Patient\ plasma)}{Activity\ control\ (Test\ plasma)} * 100$$

ANNEXURE 6 – DATA ABSTRACTION FORM

CHANGING EPIDEMIOLOGY OF ADULT POISONINGS IN A TERTIARY CARE HOSPITAL WITH EMPHASIS ON PESTICIDE, PLANT POISONINGS AND DRUGS USING THE CMC POISON DATABASE

Instruction: The Poison Control Centre database will be reviewed and the data abstraction form will be filled.

1) **Name:**
2) **Hospital number:**
3) **Age:**
4) **Sex:**
5) **Residence (by district):** Vellore / Thiruvannamalai / Krishnagiri / Chittoor / Kadapa / Others
6) **Occupation:** Farmer / Student / Labourer / Coolie / Housewife / Business / Teacher / Others
7) **Mode of poisoning:** Accidental / Deliberate self-harm / Homicide / Not known
8) **Date of consumption:**
    a. Month
    b. Year
9) **Name of the compound:**
10) **Class of the compound:**
    a. **Pesticide**
        i. ***Based on toxicity:*** Class Ia / Ib / II / III / Obsolete / Unlikely to be harmful / Unknown

ii. ***Based on category:*** Organophosphates / Organochlorine / Carbamate/ Pyrethroid / OP + Pyrethroid / Other

b. **Plant:** Oleander / Oduvanthalai / Others

c. **Drug: Drug class**

d. **Corrosive:** Phenol/Lysol / HCl / H2SO4 / Super vasmol / Others

e. **Rodenticide:** Zinc phosphide / Aluminium phosphide / Coumarins / Yellow phosphorus

f. **Unknown**

g. **Mixed**

h. **Others**

11) Status of admission: Ward admission / ICU admission / Casualty admission only

12) Discharge status: Dead / Alive / Discharge against medical advice

DATA SHEET

idno	case	age	sex	preg	marital	edu	prof	compid	compmthd	compname	comppct	comptype	compquan
1	1	20	2	1	1	2	1	1	3	dichlorvos	76	1	20
2	1	34	2	1	1	1	1	1	3	phorate	10	2	10
3	2	26	1		2	2	2	0					
4	1	34	1		2	2	2	1	3	quinalphos	25	2	60
5	1	24	2	1	1	1	1	1	2	methylpara	2	1	100
6	1	20	2	2	1	2	1	1	3	profenofos	40	3	20
7	1	26	1		1	2	1	1	3	profenofos	40	3	50
8	1	15	2	1	2	2	1	1	3	monocroto	36	1	20
9	1	27	1		1	2	2	1	3	dimethoate	35	1	50
10	1	27	1		2	2	2	1	3	monocroto	36	1	20
11	1	17	2	1	2	1	1	1	3	monocroto	36	1	20
12	1	24	2	1	1	1	1	1	3	dimethoate	35	1	75
13	1	19	2	1	2	2	3	1	3	quinalphos	25	2	200
14	1	25	2	1	2	1	1	1	2	profenofos	50	3	100
15	1	17	2	1	2	1	1	1	3	profenofos	40	3	250
16	1	19	1		1	1	1	1	2	dimethoate	30	1	100
17	1	20	2	1	2	1	1	1	3	monocroto	36	1	50
18	1	19	1		2	1	1	1	3	dimethoate	30	1	75
19	2	46	1		1	1	1	0					
20	1	55	1		1	1	1	1	2	triazophos	35	2	25
21	1	45	1		1	1	1	1	3	chlorpyrifo	40	2	15
22	1	17	2	1	1	2	1	1	3	chlorpyrifo	20	2	150
23	1	45	2	1	1	1	1	1	3	triazophos	40	2	100
24	1	23	1		2	2	1	1	3	phorate	10	2	50
25	2	22	1		2	2	1	0					
26	1	28	1		1	1	1	1	3	monocroto	30	1	10
27	2	64	2	1	1	1	1	0					
28	1	24	2		1	2	1	1	3	triazophos	40	2	50
29	1	33	2	1	1	2	1	1	3	malathion	5	1	100
30	1	37	1		1	1	2	1	3	triazophos	35	2	250

comppyre	pyrename	doc	toc	toc1	fmcd	fmct	fmct1	cmcd	cmct	cmct1	timeint1	timeint2
0		14/05/2015	20	0	14/05/2015	21	15	14/05/2015	21	15	1	1
0		16/05/2015	21	0	16/05/2015	21	45	17/05/2015	9	40	1	13
		20/05/2015	5	0	20/05/2015	7	30	20/05/2015	8	40	2.5	3.6
0		22/05/2015	19	45	22/05/2015	20	40	22/05/2015	20	40	1	1
0		22/05/2015	18	30	22/05/2015	21	15	22/05/2015	21	15	3	3
1	cypermeth	28/05/2015	20	0	28/05/2015	21	50	28/05/2015	21	50	2	2
1	cypermeth	28/05/2015	20	0	28/05/2015	21	50	28/05/2015	21	50	2	2
0		31/05/2015	16	0	31/05/2015	21	0	31/05/2015	22	15	5	6.2
0		04/06/2015	19	0	04/06/2015	23	50	04/06/2015	23	50	5	5
0		10/06/2015	8	30	10/06/2015	9	0	10/06/2015	21	45	0.5	13.2
0		11/06/2015	7	0	11/06/2015	8	0	11/06/2015	12	20	1	5.3
0		12/06/2015	17	0	12/06/2015	18	0	12/06/2015	20	50	1	4
0		12/06/2015	11	0	12/06/2015	12	0	12/06/2015	21	30	1	10.5
0		13/06/2015	20	0	14/06/2015	6	35	14/06/2015	6	35	10.5	10.5
1	cypermeth	14/06/2015	7	0	14/06/2015	8	0	14/06/2015	18	40	1	11.6
0		14/06/2015	11	30	14/06/2015	12	0	14/06/2015	18	20	0.5	7
0		14/06/2015	19	30	14/06/2015	20	30	15/06/2015	11	0	1	15.5
0		25/06/2015	10	0	25/06/2015	16	0	26/06/2015	15	0	4	29
		28/06/2015	19	0	28/06/2015	23	0	29/06/2015	12	10	4	5
1	deltamethr	30/06/2015	14	0	30/06/2015	15	0	30/06/2015	15	30	1	1.5
1	cypermeth	07/07/2015	21	0	07/07/2015	21	30	07/07/2015	23	50	0.5	3
0		08/07/2015	11	0	08/07/2015	11	20	08/07/2015	16	40	0.4	5.7
0		10/07/2015	10	0	10/07/2015	11	0	10/07/2015	22	0	1	12
0		25/07/2015	18	30	25/07/2015	19	30	25/07/2015	23	0	1	4.5
		26/07/2015	20	0	26/07/2015	22	0	26/07/2015	22	0	2	2
0		29/07/2015	14	30	29/07/2015	17	30	29/07/2015	17	30	3	3
		03/08/2015	12	0	03/08/2015	13	30	03/08/2015	14	45	1.5	2.7
0		12/08/2015	9	0	12/08/2015	10	30	12/08/2015	13	30	1.5	4.5
0		14/08/2015	8	0	14/08/2015	9	20	14/08/2015	9	20	1.3	1.3
1	deltamethr	13/08/2015	15	30	13/08/2015	17	0	14/08/2015	1	0	1.5	9.5

tout	toutskin	toutemes	toutglav	toutatr	atrdose	toutpam	pamdose	toutint	toxisal	toxilac	toxiurin	toxidef	toxivom
0										1	0	0	1
1	0	1	1	1	99	1	99			0	0	0	1
1	0	0	0	0		0				1	0	1	1	1
0										1	0	0	0	0
0										1	0	0	0	1
0										1	0	0	0	1
0										1	0	0	0	1
1	0	0	0	1	99	0			1	1	0	1	0	1
0										1	0	0	0	1
1	0	0	1	1		0			1	1	0	1	0	0
1	0	1	1	1	99	0			1	1	0	0	0	1
1	0	1	1	1	4	0			0	1	1	0	0	1
1	0	1	1	1	8	1	99		0	1	0	1	0	1
0										1	0	0	0	1
1	0	0	1	1	99	1	99		0	1	1	1	0	1
1	0	0	1	1	12	0			0	1	1	0	0	1
1	0	1	1	1	99	0			1	1	1	1	0	1
1	0	0	1	0		0			0	1	0	0	0	1
1	0	0	1	1	2	1	0.5		0	1	0	0	0	1
1	0	0	1	0		0			0	1	0	0	0	1
1	0	0	1	1	99	0			0	1	0	0	0	0
1	0	0	1	1	20	0			0	0	0	0	0	1
1	0	0	1	1	99	0			0	1	0	0	1	1
1	0	0	1	1	99	1	99		0	1	1	0	0	1
0										1	0	0	0	1
0										1	0	0	0	1
1	0	0	1	1	15	0			0	0	0	1	0	1
1	0	0	1	1	99	1	2		0	1	1	1	1	1
0										1	0	0	0	1
1	0	0	1	1	99	0			0	1	0	1	0	1

toxisz	toxibrea	toxifasc	toxialts	admgcs	admpup	admpr	admbp	admbp1	admrr	admsat	admgrbs	presvom	presdiar
0	0	0	0	015	3	102	120	80	20	100	204	1	0
0	1	1	0	15	3	99	120	80	34	100	222	1	0
0	0	0	0	15	1	100	170	90	20	100	139	1	1
0	1	0	1	3	1	109	100	60	99	80	150	0	0
0	0	0	0	15	3	60	110	60	20	90	162	1	0
0	0	0	0	15	3	84	120	80	24	97	130	1	0
0	0	0	0	15	1	86	120	70	24	99	130	1	0
1	1	0	0	7T	1	100	100	60	30	100	269	1	0
0	0	0	0	15	3	80	94	60	24	97	170	1	0
1	0	0	0	10T	1	86	100	60	98	96	143	1	0
0	0	0	1	3T	3	102	110	80	98	100	284	1	0
0	1	0	1	7	1	125	100	60	8	80	151	1	0
0	0	0	0	15	3	106	100	70	20	97	138	1	0
0	1	1	0	15	3	112	100	60	20	97	158	1	0
0	1	0	0	15	2	140	100	70	26	98	348	1	0
0	0	0	1	14	2	126	120	80	16	97	175	1	0
1	0	1	1	5T	1	84	100	60	98	99	200	0	0
0	0	1	0	3	1	152	90	60	99	70	193	1	0
0	0	0	0	15	3	108	120	80	20	99	148	1	0
0	0	0	0	15	1	130	120	80	22	86	177	1	0
0	0	0	0	15	3	110	110	80	20	96	95	1	0
0	0	0	0	13	2	78	110	80	21	96	98	1	0
0	0	0	1	12	1	110	110	60	24	90	216	1	0
0	0	0	0	15	3	104	110	70	24	98	92	1	0
0	1	1	1	3	3	84	140	90	99	64	192	1	0
0	0	0	0	15	3	110	120	80	20	96	170	0	0
0	0	0	0	14	3	102	90	60	22	96	159	1	0
0	0	0	1	14	3	100	100	70	26	98	229	1	0
0	0	0	0	15	3	75	100	70	24	95	94	1	0
0	1	0	1	15	1	112	100	60	44	90	194	0	0

presabpain	pressal	pressweat	presuri	presdrow	presbrea	pressz	presbleed	presgiddi	presalts	presagit	presasym	signdiap	signpup
1	1	1	0	0	0	0	0	0	0	0	0	1	3
0	0	0	0	0	1	0	0	0	0	0	0	0	2
0	1	1	1	0	0	0	0	0	0	0	0	1	1
0	1	0	0	0	1	0	0	0	1	0	0	0	1
0	1	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	1	0	0	0	0	0	0	0	0	0	0	0	1
0	1	0	1	1	1	1	0	0	1	0	0	0	1
0	1	0	0	0	0	0	0	0	0	0	0	0	3
0	1	1	1	0	0	1	0	0	0	0	0	0	1
0	1	0	0	1	0	0	0	0	1	0	0	0	3
0	1	1	0	0	1	0	0	0	1	1	0	1	1
0	1	0	0	0	0	0	0	0	0	0	0	0	3
0	1	0	0	0	0	0	0	0	0	1	0	0	2
0	1	1	0	1	1	0	0	0	0	1	0	1	7
0	1	1	0	0	0	0	0	0	1	1	0	1	6
0	0	0	0	1	1	1	0	0	1	0	0	0	1
0	1	0	0	0	1	0	0	0	1	0	0	0	1
0	1	1	0	0	0	1	0	0	0	0	0	1	4
0	1	1	0	0	0	0	0	0	0	0	0	1	1
0	0	0	0	1	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	1	0	0	6
0	1	1	0	0	0	0	0	0	1	0	0	1	1
0	1	0	0	0	0	0	0	0	0	0	0	0	5
0	1	0	0	1	1	0	0	0	1	0	0	0	3
0	1	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	1	1	0	0	0	0	0	0	0	0	2
0	1	0	1	1	0	0	0	0	0	0	0	0	5
0	1	0	0	0	0	0	0	0	0	0	0	1	4
0	1	0	0	0	1	0	0	0	0	0	0	0	1

signcrept	signsal	signfroth	signfasc	signsbc	signpb	signabd	signfever	severity	hr1	bp1	bp2	gcs1	gcs2
0	1	0	0	41	0	0	0	2	102	120	80	15	
0	0	0	1	21	0	0	1	3	99	120	80	15	
1	1	1	0	0	0	0	0	3	110	130	70	10	T
1	1	0	0	0	1	0	0	4	106	100	60	5	T
0	1	0	0	5	0	0	0	3	60	110	60	15	
0	0	0	0	99	0	0	0	2	84	120	80	15	
0	1	0	0	24	0	0	0	2	86	120	70	15	
1	1	0	0	99	0	0	0	4	100	120	70	7	T
0	0	0	0	0	0	0	0	2	80	94	60	15	
0	0	0	1	99	0	0	0	4	86	100	60	10	T
0	1	0	0	99	0	0	0	4	102	110	80	3	T
1	1	0	0	0	0	0	0	4	125	100	60	7	
0	1	0	0	24	0	0	0	2	106	100	70	15	
1	1	0	1	17	0	0	0	4	112	100	60	15	
1	1	1	0	0	0	0	0	4	140	100	70	15	
0	1	0	0	15	0	0	0	3	126	100	60	14	
0	0	0	1	99	0	0	0	4	84	100	60	5	T
1	1	0	1	99	1	0	0	4	152	90	60	3	
1	1	1	0	0	0	0	0	4	108	120	80	15	
0	1	0	0	20	0	0	0	2	130	120	80	15	
0	0	0	0	0	0	0	0	2	100	110	80	15	
0	0	0	0	30	0	0	0	2	122	100	70	13	
0	1	0	0	99	0	0	0	3	102	110	60	12	
0	1	0	0	30	0	0	0	2	104	110	70	15	
1	1	1	1	99	1	0	0	4	84	140	90	3	
0	1	0	0	16	0	0	0	2	110	120	80	15	
0	0	0	1	14	0	0	0	3	102	90	60	14	
1	1	0	0	13	0	0	0	3	100	100	70	14	
0	1	0	0	20	0	0	0	2	75	100	70	15	
1	1	0	1	0	0	0	0	4	110	100	60	15	

should1	elbow1	hand1	hip1	nhf1	respweak1	ventmode1	tidal1	ps1	peep1	sbc1	miosis1	crepts1	sal1	
5	5	1	5	50	0	1					41	0	0	1
5	5	1	5	0	0	1					21	1	0	0
4+	4+	1	4+	4	1	3	450	12	6		0	1	1	1
3	3	2	3	0	1	3	550	15	6		99	1	1	1
4	4	1	4	2	0	1					5	0	0	0
5	5	1	5	99	0	1					99	0	0	0
5	5	1	5	40	0	1					24	0	0	0
2	2	2	2	0	1	3	320	15	5		0	1	1	1
5	5	1	5	40	0	1					20	0	0	0
4	4	1	4	3	1	3	400	15	5		99	1	0	0
2	2	2	2	99	1	3	350	15	7		99	0	0	0
3	3	1	3	5	1	3	300	15	10		99	1	1	1
5	5	1	5	60	0	1					24	0	0	0
3	3	2	3	10	1	3	300	15	6		17	0	1	1
4	4	1	4	2	1	3	300	15	5		0	0	1	1
5	5	1	5	16	0	1					15	0	0	1
2	2	2	2	99	1	3	360	15	7		99	1	0	0
1	1	2	1	0	1	3	400	13	5		0	1	1	1
4	4	1	4	0	1	3	400	15	5		0	0	1	1
5	5	1	5	25	0	1					20	1	0	0
5	5	1	5	15	0	1					20	0	0	0
5	5	1	5	20	0	1					30	0	0	0
4+	4+	1	4+	5	0	1					99	1	0	1
5	5	1	5	40	0	1					30	0	0	0
1	1	2	1	99	1	3	400	15	8		99	0	1	1
5	5	1	5	20	0	1					16	0	0	1
5	5	1	5	99	0	1					14	0	0	0
5	5	1	5	99	0	1					13	0	1	1
5	5	1	5	45	0	1					20	0	0	1
3	3	1	3	1	1	3	400	15	5		0	1	1	1

diarr1	cholcris1	atrbolus1	atrinf1	atrtotal1	sed1	hr2	bp3	bp4	gcs3	gcs4	should2	elbow2	hand2	
0	1	0	0	0	0	90	120	80	15		5	5		1
0	1	4.8	111	115.8	0	100	130	90	15		4+	4+		1
0	1	39	24	63	0	90	120	60	15		5	5		1
0	1	3.6	31	34.6	1	90	130	80	2	T	1	1		2
0	1	2.4	20	22.4	0	100	110	60	2	T	1	1		2
0	1	0	0	0	0	98	90	60	15		5	5		1
0	1	1.8	18	19.8	0	80	120	60	15		5	5		1
0	1	17.2	73.5	90.7	1	140	130	90	9	T	2	2		2
0	1	1.8	0	1.8	0	140	110	70	15		5	5		1
0	1	24.4	114	138.5	1	101	130	80	7	T	4	4		1
0	1	5	23	28	1	97	110	60	7	T	3	3		1
0	1	6	24.3	30.3	1	108	100	50	10	T	3	3		1
0	1	4.8	0	4.8	0	100	100	70	15		5	5		1
0	1	39.3	98	137.3	0	118	100	60	10	T	3	3		2
0	1	2.4	55.5	57.9	0	90	120	70	10	T	4+	4+		1
0	1	1.2	8.5	9.7	0	106	100	60	15		5	5		1
0	1	252	426.5	678.5	1	87	110	60	2	T	1	1		2
0	1	124	203	327	1	100	120	70	10	T	3	3		2
0	1	17.4	137	154.4	0	109	150	90	8	T	1	1		2
0	1	0.6	28	28.6	0	118	120	80	15		5	5		1
0	1	2.4	24	26.4	0	102	110	80	14		5	5		1
0	0	0	0	0	0	126	110	80	15		5	5		1
0	1	1.8	9	10.8	1	100	130	70	14		4+	4+		1
0	0	0	0	0	0	100	110	70	15		5	5		1
0	1	1.2	44	45.2	1	105	130	80	8	T	1	1		2
0	0	0	0	0	0	100	120	80	15		5	5		1
0	1	1.8	28.5	30.3	0									
0	1	3.6	36	39.6	0	128	104	70	14		5	5		1
0	1	0.6	12	12.6	0	87	110	70	15		5	5		1
0	1	16	44	60	0	100	130	90	9	T	3	3		1

hip2	nhf2	respweak2	ventmode2	tidal2	ps2	peep2	sbc2	miosis2	crepts2	sal2	diarr2	cholcris2	atrbolus2
5	50	0	1					29	0	0	0	0	0
4+	0	0	1					25	0	0	0	1	0
5	25	0	1					27	0	0	0	0	0
1	99	0	3	480	15	5		99	0	0	0	1	0.6
1	99	1	3	300	15	7		99	0	0	0	1	0.6
5	20	0	1					13	0	0	0	0	0.6
5	60	0	1					50	0	0	0	0	0
2	0	1	3	320	15	6		99	0	0	0	1	0
5	40	0	1					21	0	0	0	1	2
4	0	1	3	400	15	5		99	0	0	0	1	0
3	0	1	3	300	15	6		99	0	0	0	1	0
3	0	1	3	300	15	5		99	0	0	0	1	0
5	60	0	1					20	0	0	1	0	1	4.2
3	4	1	3	315	15	6		99	0	0	0	0	1	0
4+	0	1	3	300	15	6		99	0	0	0	0	1	0
5	60	0	1					36	0	0	0	0	1	0
1	99	1	3	350	15	7		99	0	0	0	0	1	0
3	0	1	3	400	13	5		99	1	0	0	0	1	0
1	0	1	3	460	13	5		99	0	0	0	0	1	0
5	30	0	1					30	0	0	0	0	1	0
5	20	0	1					15	0	0	0	0	1	0
5	20	0	1					30	0	0	0	0	0	0
4+	10	0	1					10	0	0	0	0	1	0
5	40	0	1					28	0	0	0	0	1	1.8
1	0	1	3	400	15	5		99	0	0	0	0	1	0
5	25	0	1					25	0	0	0	0	0	0
5	40	0	1					20	0	0	0	0	1	0
5	25	0	1					20	0	1	1	0	1	0
3	0	1	3	400	15	7		99	0	0	0	0	1	0

atrinf2	atrtotal2	sed2	hr3	bp5	bp6	gcs5	gcs6	should3	elbow3	hand3	hip3	nhf3	respweak3
0	0	0	90	120	80	15		5	5		1 5	50	0
66	66	0	80	100	70	10	T	4+	4+		1 4+	5	1
0	0	0	80	140	80	15		5	5		1 5	35	0
256	256.6	1	110	120	70	6	T	2	2		2 2	0	1
183	183.6	1	110	110	70	9	T	3	3		2 3	0	1
0	0.6	0	90	100	60	15		5	5		1 5	20	0
4	4	0	80	120	70	15		5	5		1 5	60	0
141	141	0	90	120	70	10	T	3	3		2 3	0	1
20.5	22.5	0	122	110	70	15		5	5		1 5	40	0
645	645	1	100	150	80	10	T	4	4		1 4	2	1
23	23	1	100	120	80	15		4+	4+		1 4+	40	0
47.7	47.7	0	95	110	70	10	T	4-	4-		1 4-	1	1
37.3	41.5	0	100	100	70	15		5	5		1 5	30	0
53	53	0	100	100	60	10	T	3	3		2 3	0	1
45	45	0	105	120	70	10	T	3	3		1 3	0	1
19.5	19.5	0	80	100	60	15		5	5		1 5	60	0
389	389	1	96	130	70	2	T	1	1		2 1	99	1
171	171	0	100	130	70	10	T	3	3		2 3	1	1
126	126	1	90	140	80	9	T	2	2		2 2	0	1
71.5	71.5	0	96	120	80	15		5	5		1 5	30	0
53	53	0	118	110	80	15		5	5		1 5	20	0
2	2	0	130	110	70	14		4+	4+		1 4+	10	1
40.2	40.2	0	104	120	70	15		4+	4+		1 4+	10	0
0	1.8	0	105	110	70	15		5	5		1 5	40	0
110	110	1	110	120	80	8	T	3	3		2 3	5	1
0	0	0	90	120	80	15		5	5		1 5	35	0
19	19	0	94	110	70	15		5	5		1 5	40	0
2	2	0	86	110	70	15		5	5		1 5	25	0
132	132	0	96	130	90	10	T	3	3		1 3	5	1

ventmode3	tidal3	ps3	peep3	sbc3	miosis3	crepts3	sal3	diarr3	cholcris3	atrbolus3	atrinf3	atrtotal3	sed3
1				40	0	0	0	0	0	0	0	0	0
2	541	10	6	99	0	0	0	0	1	0	0	0	0
1				37	0	0	0	0	0	0	0	0	0
2	423	15	5	99	0	0	0	0	1	0	85	85	1
3	300	15	5	99	0	0	0	0	1	0	19	19	1
1				20	0	0	0	0	0	0	0	0	0
1				50	0	0	0	0	0	0	0	0	0
2	292	15	6	99	0	0	0	0	1	0	59	59	0
1				20	0	0	0	0	0	0	6.5	6.5	0
3	400	15	5	99	0	0	0	0	1	0	69	69	0
1				20	0	0	0	0	0	0	0	0	0
3	300	15	5	99	0	0	0	0	1	0	36	36	0
1				20	0	0	1	0	1	0	25	25	0
3	285	15	6	99	0	0	0	0	1	0	0	0	0
3	300	15	5	99	0	0	0	0	1	0	16	16	0
1				40	0	0	0	0	0	0	24	24	0
3	350	15	7	99	0	0	0	0	1	0	545	545	1
2	450	9	5	99	0	0	0	0	1	0	74	74	0
3	450	15	6	99	0	0	0	0	0	0	3	3	0
1				30	0	0	0	0	1	0	75	75	0
1				15	0	0	0	0	1	0	48	48	0
1				6	0	0	0	0	0	0	0	0	0
1				10	0	0	0	0	1	0	75	75	0
1				30	0	0	0	0	0	2.4	0	2.4	0
3	400	15	5	99	0	0	0	0	1	0	52	52	0
1				25	0	0	0	0	0	0	0	0	0
1				20	0	0	0	0	0	0	3	3	0
1				30	0	0	0	0	0	0	0	0	0
2	500	15	7	99	0	0	0	0	1	0	44	44	0

hr4	bp7	bp8	gcs7	gcs8	should4	elbow4	hand4	hip4	nhf4	respweak4	ventmode4	tidal4	ps4	
86	120	80	15		5	5	1	5	50	0	1			
80	80	50	10	T	4+	4+	1	4+	15	1	3	500		8
80	130	80	15		5	5	1	5	40	0	1			
110	120	80	7	T	3	3	2	3	0	1	2	375		10
90	130	80	15		4-	4-	1	4-	5	0	1			
85	100	60	15		5	5	1	5	20	0	1			
80	120	70	15		5	5	1	5	60	0	1			
120	130	70	10	T	3	3	1	3	1	1	3	323		18
100	110	70	15		5	5	1	5	40	0	1			
90	120	70	10	T	4	4	1	4	2	1	3	400		15
90	120	80	15		5	5	1	5	60	0	1			
120	100	50	10	T	4-	4-	1	4-	4	0	3	310		15
98	100	70	15		4	4	1	4	15	0	1			
126	100	60	10	T	2	2	2	2	0	1	3	470		14
105	130	80	10	T	3	3	1	3	0	1	3	300		12
80	100	60	15		5	5	1	5	60	0	1			
107	130	70	2	T	1	1	2	1	99	1	3	350		15
70	140	70	10	T	4	4	1	4	10	1	2	450		10
100	140	80	9	T	2	2	2	2	0	1	3	450		15
90	120	80	15		5	5	1	5	40	0	1			
94	110	80	15		5	5	1	5	30	0	1			
106	100	70	15		5	5	1	5	20	0	1			
102	120	70	10	T	2	2	1	2	5	1	3	360		15
106	110	70	15		5	5	1	5	40	0	1			
100	120	80	10	T	5	5	1	5	20	0	2	407		15
88	100	70	14		5	5	1	5	40	0	1			
85	110	70	15		5	5	1	5	40	0	1			
81	140	80	10	T	5	5	1	5	20	0	2	500		10

peep4	sbc4	miosis4	crepts4	sal4	diarr4	cholcris4	atrbolus4	atrinf4	atrtotal4	sed4	hr5	bp9	bp10
	40	0	0	0	0	0	0	0	0	0	85	120	80
9	99	0	0	0	0	0	0	0	0	0	85	110	70
	40	0	0	0	0	0	0	0	0	0			
5	99	0	0	0	0	0	0	0	0	1	80	110	70
	7	0	0	0	0	0	0	0	0	0	106	110	70
	20	0	0	0	0	0	0	0	0	0			
	50	0	0	0	0	0	0	0	0	0	80	120	80
6	99	0	0	0	0	1	0	36	36	0	120	120	70
	25	0	0	0	0	0	0	0	0	0	90	110	70
5	99	0	0	0	0	0	0	1	1	0	100	120	70
	25	0	0	0	0	0	0	0	0	0	84	120	80
5	99	0	0	0	0	0	0	0	0	0	100	100	60
	5	0	0	0	0	0	0	1	1	0	80	100	70
9	99	0	0	0	0	0	0	0	0	0	88	100	60
5	99	0	0	0	0	0	0	0	0	0	88	100	70
	50	0	0	0	0	0	0	5.5	5.5	0	58	100	60
7	99	0	0	0	0	1	0	460	460	1	100	130	70
5	99	0	0	0	0	0	0	20	20	0	85	130	90
9	99	0	0	0	0	0	0	0	0	0	100	120	80
	30	0	0	0	0	1	0	29	29	0	96	120	80
	40	0	0	0	0	1	0	21	21	0	98	110	80
	60	0	0	0	0	0	0	0	0	0	108	100	70
5	99	0	0	0	0	0	0	84	84	0	96	120	70
	30	0	0	0	0	0	1.8	0	1.8	0	96	110	70
5	99	0	0	0	0	0	0	0	0	0	90	120	80
	26	0	0	0	0	0	0	0	0	0	88	100	70
	30	0	0	0	0	0	0	0	0	0	80	110	70
6	99	0	0	0	0	1	0	24	24	0	80	100	70

gcs9	gcs10	should5	elbow5	hand5	hip5	nhf5	respweak5	ventmode5	tidal5	ps5	peep5	sbc5	miosis5	
	15	5	5	1	5	50	0	1					40	0
	10 T	3	3	1	3	0	1	2	300	6	7		99	0
	7 T	2	2	2	2	2	1	3	450	15	8		99	0
	15	4+	4+	1	4+	15	0	1					10	0
	15	5	5	1	5	60	0	1					50	0
	10 T	3	3	1	3	1	1	3	300	15	6		99	0
	15	5	5	1	5	40	0	1					25	0
	10 T	5	5	1	5	40	0	2	381	15	5		99	0
	15	5	5	1	5	60	0	1					30	0
	10 T	4	4	1	4	15	0	2	371	15	5		99	0
	15	5	5	1	5	15	0	1					10	0
	10 T	4-	4-	1	4-	5	1	3	280	15	7		99	0
	10 T	4-	4-	1	4-	5	1	3	306	12	5		99	0
	15	5	5	1	5	60	0	1					54	0
	2 T	1	1	2	1	99	1	3	400	15	7		99	0
	10 T	4+	4+	1	4+	30	1	2	400	10	5		99	0
	9 T	2	2	2	2	0	1	3	400	15	9		99	0
	15	5	5	1	5	40	0	1					30	0
	15	5	5	1	5	30	0	1					40	0
	15	5	5	1	5	20	0	1					60	0
	10 T	4+	4+	1	4+	5	1	3	360	15	5		99	0
	15	5	5	1	5	40	0	1					30	0
	15	5	5	1	5	45	0	1					10	0
	15	5	5	1	5	40	0	1					26	0
	15	5	5	1	5	40	0	1					30	0
	15	5	5	1	5	20	0	1					99	0

crepts5	sal5	diarr5	cholcris5	atrbolus5	atrinf5	atrtotal5	sed5	insyn	insyndur	icu	icudur	vent	ventind
	0	0	0	0	0	0	0	0	0		0		0	
	0	0	0	0	0	0	0	0	1	3	1	6	1	2
									0		1	2	1	3
	0	0	0	0	0	0	0	1	1	5	1	9	1	1
	0	0	0	0	0	0	0	0	0		1	3	1	2
									0		0		0	
	0	0	0	0	0	0	0	0	0		0		0	
	0	0	0	0	0	0	0	0	1	9	1	16	1	3
	0	0	0	0	0	0	0	0	0		0		0	
	0	0	0	0	0	0	0	0	0		1	7	1	1
	0	0	0	0	0	0	0	0	0		1	4	1	1
	0	0	0	0	0	0	0	0	0		1	6	1	3
	0	0	0	0	0	0	0	0	0		0		0	
	0	0	0	0	0	0	0	0	1	8	1	11	1	3
	0	0	0	0	0	0	0	0	1	10	1	16	1	2
	0	0	0	0	0	9.5	9.5	0	0		0		0	
	0	0	0	0	0	0	0	1	0		1	5	1	1
	0	0	0	0	0	0	0	0	1	2	1	7	1	1
	0	0	0	0	0	0	0	0	1	17	1	19	1	3
	0	0	0	0	0	0	0	0	0		0		0	
	0	0	0	0	0	5	5	0	0		0		0	
	0	0	0	0	0	0	0	0	1	1	1	2	0	
	0	0	0	0	0	10.5	10.5	0	1	11	1	8	1	2
	0	0	0	0	1.2	0	1.2	0	0		0		0	
	0	0	0	0	0	0	0	0	0		1	5	1	3
									0		0		0	
									0		0		1	2
	0	0	0	0	0	0	0	0	0		0		0	
	0	0	0	0	0	0	0	0	0		0		0	
	0	0	0	0	0	7	7	0	1	11	1	10	1	2

intday	ventdur	trach	cardarr	resparr	infec	infec1	infec2	atrdel	inotrop	lowgccs	cmcglav	cmcchar	cmcatr
			0	0	0				0	0	0	1	1	0
2	5	0	0	0	1	3			0	1	0	1	0	1
1	1.2	0	0	0	0				0	0	0	1	0	1
1	8	0	0	0	1	1			0	0	5	1	1	1
2	2	0	0	0	0				0	0	1	1	0	1
			0	0	0				0	0	0	1	1	1
			0	0	0				0	0	0	1	0	1
1	14	0	0	0	1	1	3		0	0	1	0	0	1
			0	0	0				0	0	0	1	1	1
1	6	0	0	0	0				0	0	2	0	0	1
1	2	0	0	0	0				0	0	2	1	1	1
1	6	0	0	0	0				0	0	1	1	0	1
			0	0	0				0	0	0	0	0	1
1	11	0	0	0	1	1			0	0	0	1	1	1
1	13	1	0	0	1	1			0	0	0	0	0	1
			0	0	0				0	0	0	1	0	1
1	5	0	0	1	1	1			0	1	5	0	0	1
2	6	0	0	0	0				0	0	1	0	0	1
1	14	1	0	0	1	1			0	0	1	1	0	0
			0	0	0				0	0	0	1	1	1
			0	0	0				0	0	0	1	1	1
			0	0	0				0	0	0	1	1	1
4	11	0	0	0	1	1			0	0	1	0	0	1
			0	0	0				0	0	0	0	0	1
1	4	0	0	0	0				0	0	1	1	1	1
			0	0	0				0	0	0	1	1	0
1	0	0	0	1	0				0	0	0	1	1	1
			0	0	1	1			1	0	0	1	1	1
			0	0	0				0	0	0	1	1	1
1	9	1	0	0	1	1			0	0	0	0	0	1

atrdur	atrdose1	death	deathcause	outcome	hospdur
0	0	0		1	5
2	181.8	0		1	9
1	63	0		1	4
3	376.2	0		1	11
3	225	0		1	7
1	0.6	0		1	5
2	23.8	0		1	5
4	326.7	0		1	19
3	30.8	0		1	5
4	853.5	0		1	8
2	51	0		1	7
3	114	0		1	8
4	72.3	0		1	6
2	190.3	1	2	2	11
3	118.9	0		1	27
6	69.2	0		1	6
4	2072.5	1	2	2	5
4	592	0		1	9
3	283.4	0		1	27
4	204.1	0		1	8
4	148.4	0		1	7
1	2	0		1	6
5	220.5	0		1	26
4	7.2	0		1	7
3	207.2	0		1	6
0	0	0		1	3
1	30.3	1	3	2	1
3	61.6	0		1	5
2	14.6	0		1	5
5	267	0		1	20

idno	preoximest	preoxime1	preoxime2	preoxime3	preoxime4	preoxime5	postoxstat	postox1
1	94.77%	40.78%	26.52%				111.11%	67.12%
2	44.33%	38.37%	22.57%	28.97%	34.37%	21.76%	191.20%	178.53%
3	22.29%	43.54%	55.67%	57.51%			144.56%	101.15%
4	91.48%	150.89%	197.18%	113.49%	295.28%	213.56%	116.33%	181.85%
5	104.65%	75.04%	94.39%	107.18%	92.41%	99.58%	135.74%	112.75%
6	249.85%	193.66%	233.47%	249.91%			294.54%	245.65%
7	37.03%	16.01%	15.21%	72.14%			51.92%	37.34%
8	0.00%	9.64%	9.02%	11.00%	0.00%	19.68%	33.38%	20.29%
9	11.00%	43.76%	99.09%	108.48%			32.51%	73.68%
10	7.54%	6.68%	7.79%	19.78%	13.18%	13.83%	11.87%	0.00%
11	194.96%	223.83%	158.49%	93.15%	74.79%	91.98%	175.80%	309.50%
12	23.39%	24.91%	11.71%	24.34%	29.24%	26.01%	22.54%	14.95%
13	50.80%	50.80%	30.00%	34.78%	35.55%	21.11%	88.36%	88.36%
14	20.47%	49.48%	7.76%	20.35%	15.52%	9.76%	22.04%	50.15%
15	19.33%	17.38%	9.10%	18.77%	32.03%	17.23%	12.04%	8.43%
16	19.57%	11.96%	17.64%	22.73%	22.01%	57.80%	19.84%	12.45%
17	16.06%	7.44%	7.78%	16.08%	19.85%		20.84%	21.91%
18	8.86%	2.69%	0.00%	1.54%	2.33%	15.39%	0.00%	10.53%
19	4.37%	1.06%	1.04%	0.00%	10.71%	0.00%	6.07%	7.05%
20	28.84%	6.04%	8.16%	0.00%	25.00%	1.08%	80.06%	29.94%
21	33.78%	2.38%	0.21%	4.05%	4.75%		79.08%	7.15%
22	26.14%	15.41%	1.79%	12.11%		16.92%	43.45%	59.42%
23	16.83%	16.83%	8.28%	5.26%	0.00%	1.97%	74.26%	74.26%
24	112.25%	101.33%	131.67%	123.36%	15.72%		115.68%	153.06%
25	24.77%	18.68%	16.41%	4.12%	12.09%		88.83%	85.16%
26	90.51%	110.45%					91.86%	121.88%
27	2.28%						5.84%	
28	10.49%	26.47%	2.87%	50.26%			112.94%	77.81%
29	100.14%	109.51%	106.42%	118.77%	103.90%	110.57%	130.68%	
30	18.60%	13.29%	0.00%	7.60%	0.00%	6.74%	107.83%	95.18%

postox2	postox3	postox4	postox5	%reactstat	%react1	%react2	%react3	%react4
57.84%				16.34%	26.34%	31.32%		
102.49%	133.10%	81.08%	95.16%	146.87%	140.16%	79.92%	104.13%	46.71%
89.84%	82.77%			122.27%	57.61%	34.17%	25.26%	
245.58%	135.56%	421.26%	255.41%	24.85%	30.96%	48.40%	22.07%	125.98%
111.51%	129.37%	132.90%	132.59%	31.09%	37.71%	17.12%	22.19%	40.49%
265.92%	214.00%			44.69%	51.99%	32.45%	-35.91%	
40.61%	86.79%			14.89%	21.33%	25.40%	14.65%	
29.18%	56.23%	0.00%	21.64%	33.38%	10.65%	20.16%	45.23%	0.00%
124.92%	129.99%			21.51%	29.92%	25.83%	21.51%	
9.52%	16.69%	0.00%	6.48%	4.33%	-6.68%	1.73%	-3.09%	-13.18%
174.50%	129.75%	90.87%	125.48%	-19.16%	85.67%	16.01%	36.60%	16.08%
10.15%	12.54%	0.00%	0.00%	-0.85%	-9.96%	-1.56%	-11.80%	-29.24%
61.62%	49.52%	43.26%	40.58%	37.56%	37.56%	31.62%	14.74%	7.71%
20.69%	0.00%	13.18%	10.53%	1.57%	0.67%	12.93%	-20.35%	-2.34%
3.84%	22.77%	12.63%	24.80%	-7.29%	-8.95%	-5.26%	4.00%	-19.40%
9.02%	23.39%	19.56%	52.87%	0.27%	0.49%	-8.62%	0.66%	-2.45%
4.79%	3.41%	7.20%		4.78%	14.47%	-2.99%	-12.67%	-12.65%
2.95%	0.44%	0.52%	11.76%	-8.86%	7.84%	2.95%	-1.10%	-1.81%
4.16%	74.00%	5.35%	5.86%	1.70%	5.99%	3.12%	74.00%	-5.36%
28.10%	25.11%	13.83%	11.37%	51.22%	23.90%	19.94%	25.11%	-11.17%
0.42%	6.94%	8.49%		45.30%	4.77%	0.21%	2.89%	3.74%
57.95%	47.32%		27.83%	17.31%	44.01%	56.16%	35.21%	
33.14%	20.23%	15.67%	7.61%	57.43%	57.43%	24.86%	14.97%	15.67%
132.08%	128.44%	32.73%		3.43%	51.73%	0.41%	5.08%	17.01%
45.68%	49.24%	31.10%		64.06%	66.48%	29.27%	45.12%	19.01%
				1.35%	11.43%			
				3.56%				
103.37%	64.18%			102.45%	51.34%	100.50%	13.92%	
114.87%		113.60%	127.26%	30.54%	-109.51%	8.45%	-118.77%	9.70%
65.31%	62.42%	38.59%	32.77%	89.23%	81.89%	65.31%	54.82%	38.59%

%react5	react durat	inh stat	inh 2	inh 3	inh 4	inh 5
	0					
73.40%	6	41.00%	59.00%	61.00%	84.00%	90.00%
	1					
41.85%	0					
33.01%	0	88.00%	94.00%	91.00%		97.00%
	0	206.00%	97.00%	99.00%	90.00%	
	2	95.00%	98.00%	87.00%	86.00%	
1.96%	1		93.00%	88.00%	90.00%	94.00%
	1	95.00%	91.00%	74.00%	88.00%	
-7.35%	0	121.00%	115.00%	112.00%	99.00%	78.00%
33.50%	0	102.00%	119.00%	107.00%		110.00%
-26.01%	0	66.00%	96.00%	78.00%	91.00%	82.00%
19.47%	2	97.00%	118.00%	105.00%	83.00%	
0.77%	0	96.00%	78.00%	97.00%	65.00%	86.00%
7.57%	0	92.00%	79.00%	78.00%	81.00%	111.00%
-4.93%	0	90.00%	99.00%	79.00%	89.00%	107.00%
0.00%	0	59.00%	93.00%	79.00%	95.00%	
-3.63%	0	85.00%	93.00%	90.00%	94.00%	92.00%
5.86%	0	82.00%	65.00%	64.00%	86.00%	82.00%
10.29%	1	88.00%	74.00%	94.00%	86.00%	87.00%
	0.5	114.00%	115.00%		120.00%	102.00%
10.91%	3	110.00%		116.00%	112.00%	90.00%
5.64%	2	88.00%	100.00%	92.00%	88.00%	91.00%
	0					
	4					
	0					
	0					
	2	89.00%	78.00%	92.00%	88.00%	
16.69%	0					
26.03%	5	72.00%	64.00%	79.00%	81.00%	86.00%